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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

CORCEPT THERAPEUTICS, INC., **Plaintiff,** **v.** **TEVA PHARMACEUTICALS USA, INC. and TEVA PHARMACEUTICALS INDUSTRIES LTD.,** **Defendants.**	**Civil Action No. 18-3632 (SDW)(CLW)** **FIRST AMENDED COMPLAINT FOR PATENT INFRINGEMENT** **(Filed Electronically)**

Plaintiff Corcept Therapeutics, Inc. ("Corcept"), by its undersigned attorneys, for its First Amended Complaint[1] against defendants Teva Pharmaceuticals USA, Inc. ("Teva USA") and Teva Pharmaceuticals Industries Ltd. ("Teva Ltd.," collectively, "Teva"), alleges as follows:

[1] Corcept files this First Amended Complaint for Patent Infringement as a matter of course pursuant to Fed. R. Civ. P. 15(a)(1)(B), as it is being filed within 21 days of service of Teva's Motion to Dismiss Pursuant to Fed. R. Civ. P. 12(b)(6) (D.I. 12), which was filed on June 15, 2018. As a result of filing the Amended Complaint, Corcept does not intend to respond to Teva's June 15 Motion to Dismiss because amendment of a complaint as of right moots a previously filed motion to dismiss. *See Harnish v. Widener Univ. Sch. Of Law*, Civ. No. 12-608, 2012 WL 2576353, at *3 (D.N.J. July 3, 2012).

Nature of the Action

1. This complaint is an action for patent infringement under the patent laws of the United States, 35 U.S.C. §100, *et seq.*, arising from Teva's filing of an Abbreviated New Drug Application ("ANDA") No. 211436 ("Teva's ANDA") with the United States Food and Drug Administration ("FDA") seeking approval to commercially market a generic version of Corcept's 300 mg mifepristone drug product ("Teva's Proposed Product") prior to the expiration of United States Patent Nos. 8,921,348 ("'348 patent"), 9,829,495 ("'495 patent"), and 9,943,526 ("'526 patent) (collectively, "the patents-in-suit") owned by Corcept.

The Parties

2. Plaintiff Corcept is a biopharmaceutical company committed to improving the lives of patients worldwide. Corcept focuses on, and heavily invests in, the discovery and development of drugs that regulate the effects of cortisol for the treatment of severe and life-threatening conditions, including Cushing's syndrome. Corcept is an industry leader for the development of orphan-status rare disease drugs, including Korlym®. Corcept is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 149 Commonwealth Dr., Menlo Park, CA 94025.

3. On information and belief, Defendant Teva USA is a corporation organized and existing under the laws of the State of Delaware, having a principal place of business at 1090 Horsham Road, North Wales, Pennsylvania 19454.

4. On information and belief, Teva Ltd. is a corporation organized and existing under the laws of Israel, having a principal place of business at 5 Basel Street, Petach Tikva, 49131, Israel.

5. On information and belief, Teva USA is a wholly-owned subsidiary of Teva Ltd.

6. On information and belief, Teva is in the business of marketing, distributing, and selling pharmaceutical drugs, including generic pharmaceutical drugs manufactured by Teva, throughout the United States, including this Judicial District.

7. On information and belief, Teva USA, in collaboration with other Teva entities, prepared and submitted Teva's ANDA and continues to collaborate in seeking FDA approval of that application.

8. On information and belief, the acts of Teva USA complained of herein were done at the direction of, with the authorization of, or with the cooperation, participation, or assistance of, or at least in part for the benefit of, Teva Ltd.

The Patents-in-Suit

9. On December 30, 2014, the United States Patent and Trademark Office ("USPTO") duly and lawfully issued the '348 patent, entitled, "Optimizing mifepristone levels in plasma serum of patients suffering from mental disorders treatable with glucocorticoid receptor antagonists" to Corcept as assignee of the inventor Joseph K. Belanoff. A copy of the '348 patent is attached hereto as Exhibit A.

10. On November 28, 2017, the USPTO duly and lawfully issued the '495 patent, entitled, "Method for differentially diagnosing ACTH dependent Cushing's Syndrome" to Corcept as assignee of the inventor Andreas G. Moraitis. A copy of the '495 patent is attached hereto as Exhibit B.

11. On April 17, 2018, the USPTO duly and lawfully issued the '526 patent, entitled "Optimizing Mifepristone Levels for Cushing's Patients" to Corcept as assignee of the inventors Joseph Belanoff and Coleman Gross. A copy of the '526 patent is attached hereto as Exhibit C.

The KORLYM® Drug Product

12. Corcept holds an approved New Drug Application ("NDA") under Section 505(a) of the Federal Food Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. § 355(a), for mifepristone tablets (NDA No. 202107), which it sells under the trade name KORLYM®. KORLYM® is an FDA-approved medication for the treatment of hyperglycemia secondary to hypercortisolism in adult patients with endogenous Cushing's syndrome who have type 2 diabetes mellitus or glucose intolerance and have failed surgery or are not candidates for surgery. The claims of the patents-in-suit cover, inter alia, methods of use and administration of mifepristone and methods of use of concurrent treatments for patients with Cushing's syndrome.

13. Pursuant to 21 U.S.C. § 355(b)(1) and attendant FDA regulations, the patents-in-suit are listed in the FDA publication, "Approved Drug Product with Therapeutic Equivalence Evaluations" (the "Orange Book"), with respect to KORLYM®.

Jurisdiction and Venue

14. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

15. This Court has personal jurisdiction over Teva USA by virtue of, inter alia, Teva USA's systematic and continuous contacts with the State of New Jersey. On information and belief, Teva USA is registered with the State of New Jersey's Division of Revenue and Enterprise Services as a business operating in New Jersey under Business Id. No. 0100250184. On information and belief, Teva USA is registered with the State of New Jersey's Department of Health as a drug "manufacturer and wholesaler" and as a drug "wholesaler" under Registration Nos. 5000583 and 5003436, respectively. On information and belief, Teva USA has employees in New Jersey facilities, including at 8 Gloria Lane, Fairfield, New Jersey 07004 and at 208 Passaic Avenue, Fairfield, New Jersey 07004. *See Indivior Inc., et al. v. Teva Pharmaceuticals*

USA, Inc., Civil Action No. 17-7115, D.I. 6 at ¶ 8. On information and belief, Teva USA also has employees in a New Jersey facility located at 400 Interpace Pkwy #3, Parsippany, New Jersey 07054. *See* TEVA PHARMACEUTICALS INDUSTRIES LIMITED, Annual Report (Form 10-K), at Ex. 10.31, Ex. 10.32 (February 12, 2018). On information and belief, Teva USA has a registered agent for service of process in New Jersey. *See Indivior Inc., et al. v. Teva Pharmaceuticals USA, Inc.,* Civil Action No. 17-7115, D.I. 6 at ¶ 7. By virtue of Teva USA's physical presence in New Jersey, this Court has personal jurisdiction over Teva USA.

16. On information and belief, Teva USA has conducted business in this Judicial District and has purposefully availed itself of this forum by, among other things, manufacturing, marketing, distributing, offering for sale, generating revenue or selling pharmaceutical products, including generic drug products, throughout the United States, including this Judicial District.

17. On information and belief, Teva USA has prepared and/or aided in the preparation and submission of ANDAs to the FDA.

18. On information and belief, this Judicial District will be a destination for the generic drug product described in Teva's ANDA.

19. On information and belief, Teva USA was sued for patent infringement in this Judicial District and did not contest personal jurisdiction in this Judicial District in at least the following cases: *Amarin Pharma, Inc., et al. v. Teva Pharmaceuticals USA, Inc.*, Civil Action No. 14-3558; *Boehringer Ingelheim Pharma GmBH & Co. KG, et al. v. Teva Pharmaceuticals USA, Inc., et al.,* Civil Action No. 14-7811; *Novo Nordisk Inc., et al., v. Teva Pharmaceuticals USA, Inc.,* Civil Action No. 14-4248; *Otsuka Pharmaceutical Co., Ltd. v. Teva Pharmaceuticals USA, Inc., et al.,* Civil Action No. 14-5878; and *United Therapeutics Corp. v. Teva Pharmaceuticals USA, Inc.,* Civil Action No. 14-5498. On information and belief, Teva USA

purposefully availed itself of the benefits of this forum by filing counterclaims in each of those actions.

20. On information and belief, Teva USA availed itself of this forum by bringing civil actions for patent infringement in this forum in at least the following cases: *Teva Pharmaceuticals USA, Inc., Teva Pharmaceutical Industries Ltd., and Teva Neuroscience, Inc. v. Sandoz Inc., et al.,* Civil Action No. 17-275 (FLW)(DEA) (D.N.J.); *Teva Pharmaceuticals USA, Inc., Teva Pharmaceutical Industries Ltd., and Teva Neuroscience, Inc. v. Dr. Reddy's Laboratories, Ltd.,* Civil Action No. 17-517 (FLW)(DEA) (D.N.J.); *Teva Neuroscience, Teva Pharmaceutical Industries Ltd., Teva Pharmaceutical USA, Inc., and Yeda Research and Development Co., Ltd. v. Dr. Reddy's Laboratories, Inc., et al.,* Civil Action No. 14-5672 (MAS)(TJB) (D.N.J.); *Teva Pharmaceuticals USA, Inc., et al. v. Dr. Reddy's Laboratories, Ltd., et al.*, Civil Action No. 15-471; and, *Teva Pharmaceuticals USA, Inc., et al. v. Synthon Pharmaceuticals, Inc., et al.,* Civil Action No. 15-472.

21. In its Offer of Confidential Access, Teva USA stated that it "irrevocably submit[ted] to and accept[ed], generally and unconditionally, the exclusive personal jurisdiction … of the U.S. District Court for the State of New Jersey [and] waive[d] its right to assert any objection or defense based on venue…."

22. This Court has personal jurisdiction over Teva Ltd. because, inter alia, it: (1) has purposely availed itself of the privilege of doing business in New Jersey, including directly or indirectly through its subsidiary, agent, and/or alter ego, Teva USA, a company registered with the State of New Jersey's Department of Health as a drug manufacturer and wholesaler; and (2) has maintained extensive and systematic contacts with the State of New Jersey, including

manufacturing, marketing, distributing, offering for sale, generating revenue or selling pharmaceutical products in New Jersey, including through, directly or indirectly, Teva USA.

23. On information and belief, Teva Ltd. was previously sued in this Judicial District and did not challenge personal jurisdiction. *See, e.g., Boehringer Ingelheim Pharma GmbH & Co., et al. v. Teva Pharmaceuticals USA, Inc., et al.,* Civil Action No. 14-7811; *Janssen Prods., L.P., et al. v . Teva Pharmaceuticals USA, Inc., et al.,* Civil Action No. 13-7576.

24. Teva Ltd. availed itself of this Court's jurisdiction by initiating litigation in this Judicial District. *See, e.g., Teva Pharmaceuticals USA, Inc., Teva Pharmaceutical Industries Ltd., and Teva Neuroscience, Inc. v. Sandoz Inc., et al.,* Civil Action No. 17-275; *Teva Pharmaceuticals USA, Inc., Teva Pharmaceutical Industries Ltd., and Teva Neuroscience, Inc. v. Dr. Reddy's Laboratories, Ltd.,* Civil Action No. 17-517; *Teva Neuroscience, Teva Pharmaceutical Industries Ltd., Teva Pharmaceutical USA, Inc., and Yeda Research and Development Co., Ltd. v. Dr. Reddy's Laboratories, Inc., et al.,* Civil Action No. 14-5672; *Teva Pharmaceuticals USA, Inc., et al. v. Dr. Reddy's Laboratories, Ltd., et al.,* Civil Action No. 15-471, *Teva Pharmaceuticals USA, Inc., et al. v. Synthon Pharmaceuticals, Inc., et al.,* Civil Action No. 15-472.

25. On information and belief, Teva USA and Teva Ltd. have worked in concert with respect to regulatory approval, manufacturing, marketing, distributing, offering for sale, or selling generic pharmaceutical products throughout the United States, including in this Judicial District.

26. On information and belief, Teva USA has acted at the direction and for the benefit of Teva Ltd., and Teva Ltd. has controlled and/or dominated Teva USA.

27. On information and belief, Teva Ltd. has prepared and/or aided in the preparation and submission of ANDAs to the FDA, including through, directly or indirectly, Teva USA.

28. This Court holds personal jurisdiction over Teva because, inter alia, Teva committed an act of patent infringement in this Judicial District under 35 U.S.C. § 271(e)(2) and sent notice of that infringement to Corcept from the State of New Jersey.

29. On information and belief, Teva's future course of conduct will lead to acts of patent infringement in New Jersey. Teva's future course of conduct will lead to foreseeable harm and injury to Corcept in New Jersey and this Judicial District.

30. Venue is proper in this Judicial District pursuant to 28 U.S.C. §§ 1391 and/or 1400(b).

Acts Giving Rise To This Suit

31. No earlier than January 31, 2018, Teva sent written notice of a Paragraph IV Certification ("Teva's 1st Notice Letter") to Corcept. According to Teva's 1st Notice Letter, Teva filed an ANDA pursuant to Section 505 of the FFDCA seeking approval to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Teva's Proposed Product before the '348 patent and '495 patent expire.

32. Teva's 1st Notice Letter alleges that the claims of the '348 patent and '495 patent are invalid and/or will not be infringed by the activities described in Teva's ANDA.

33. On information and belief, in connection with the filing of its ANDA as described above, Teva provided a written certification to the FDA, as called for by Section 505 of the FFDCA, 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Teva's 1st Paragraph IV Certification"), alleging that the claims of the '348 patent and '495 patent are invalid, unenforceable, and/or will not be infringed by the activities described in Teva's ANDA.

34. No earlier than May 14, 2018, Teva sent written notice of a second Paragraph IV Certification ("Teva's 2nd Notice Letter") to Corcept. According to Teva's 2nd Notice Letter Teva filed an ANDA pursuant to Section 505 of the FFDCA seeking approval to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Teva's Proposed Product before the '526 patent expires.

35. Teva's 2nd Notice Letter alleges that the claims of the '526 patent are invalid and/or will not be infringed by the activities described in Teva's ANDA.

36. On information and belief, in connection with ANDA No. 211436, Teva provided a written certification to the FDA, as called for by Section 505 of the FFDCA, 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Teva's 2nd Paragraph IV Certification"), alleging that the claims of the '526 patent are invalid, unenforceable, and/or will not be infringed by the activities described in Teva's ANDA.

37. On information and belief, following FDA approval of Teva's ANDA, Teva USA and Teva Ltd will work in concert with one another to make, use, offer to sell, or sell Teva's Proposed Products throughout the United States, or import such generic products into the United States.

Count I: Infringement of the '348 Patent

38. Corcept repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

39. Teva's submission of its ANDA and accompanying Paragraph IV Certifications to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Teva's Proposed Products, prior to the expiration of the '348 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

40. A justiciable controversy exists between the parties hereto as to the infringement of the '348 patent.

41. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will infringe one or more claims of the '348 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Teva's Proposed Products in the United States.

42. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will induce infringement of one or more claims of the '348 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, upon FDA approval of Teva's ANDA, Teva will intentionally encourage acts of direct infringement with knowledge of the '348 patent and knowledge that its acts are encouraging infringement. For example, according to Teva's 1st Notice Letter, Teva will instruct prescribers to increase the dose of Teva's Proposed Products "based on a clinical assessment of tolerability and degree of improvement in Cushing's syndrome manifestations." On information and belief, Teva will also instruct prescribers that, in the absence of improvements in Cushing's syndrome manifestations, prescribers should consider measuring a trough plasma KORLYM level to guide additional titration. On information and belief, Teva will further instruct prescribers that in a study of patients with Cushing's syndrome, all patients who reached a trough KORLYM concentration of at least 2200 ng/ml had significant clinical improvement based on a centrally-adjudicated, eight-category assessment of clinical response. These instructions will cause the prescribers to perform the steps claimed in the '348 patent.

43. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will contributorily infringe one or more claims of the '348 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United

States. On information and belief, Teva knew and knows that Teva's Proposed Product is designed for a use that infringes one or more claims of the '348 patent, and Teva's Proposed Product lacks a substantial non-infringing use. For example, according to Teva's 1st Notice Letter, "FDA has received an Abbreviated New Drug Application ('ANDA') from Teva for Mifepristone Tablets, 300 mg.... The ANDA was submitted under 21 U.S.C. § 355(j)(1) and (2)(A), and contains Paragraph IV certifications to obtain approval to engage in the commercial manufacture, use or sale of Mifepristone Tablets, 300 mg, before the expiration of U.S. Patent Nos. 8,921,348 and 9,829,495...."

44. Failure to enjoin Teva's infringement of the '348 patent will substantially and irreparably damage Corcept.

45. Corcept does not have an adequate remedy at law.

Count II: Infringement of the '495 Patent

46. Corcept repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

47. Teva's submission of its ANDA and accompanying Paragraph IV Certifications to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Teva's Proposed Product, prior to the expiration of the '495 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

48. A justiciable controversy exists between the parties hereto as to the infringement of the '495 patent.

49. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will infringe one or more claims of the '495 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States.

50. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will induce infringement of one or more claims of the '495 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, upon FDA approval of Teva's ANDA, Teva will intentionally encourage acts of direct infringement with knowledge of the '495 patent and knowledge that its acts are encouraging infringement. For example, according to Teva's 1st Notice Letter, Teva will instruct prescribers to determine whether a patient with Cushing's syndrome is a candidate for surgery. The '495 patent covers a method for differentially diagnosing ectopic ACTH syndrome and Cushing's disease, which prescribers will use to determine whether a patient with Cushing's syndrome is a candidate for surgery. The '495 patent explains that "[c]orrect differential diagnosis between [] Cushing Disease and ectopic ACTH syndrome is important for endocrinologists to recommend transphenoidal surgery or appropriate imaging to identify source of the ectopic ACTH secretion." '495 patent at 2:35-39. Teva's instruction to prescribers to determine whether a patient is a candidate for surgery will cause the prescribers to differentially diagnose ectopic ACTH syndrome and Cushing's disease according to the methods claimed in the '495 patent.

51. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will contributorily infringe one or more claims of the '495 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, Teva knew and knows that Teva's Proposed Product is designed for a use that infringes one or more claims of the '495 patent, and Teva's Proposed Product lacks a substantial non-infringing use. For example, according to Teva's 1st Notice Letter, "FDA has received an Abbreviated New Drug Application ('ANDA') from Teva for

Mifepristone Tablets, 300 mg.... The ANDA was submitted under 21 U.S.C. § 355(j)(1) and (2)(A), and contains Paragraph IV certifications to obtain approval to engage in the commercial manufacture, use or sale of Mifepristone Tablets, 300 mg, before the expiration of U.S. Patent Nos. 8,921,348 and 9,829,495...."

52. Failure to enjoin Teva's infringement of the '495 patent will substantially and irreparably damage Corcept.

53. Corcept does not have an adequate remedy at law.

Count III: Infringement of the '526 Patent

54. Corcept repeats and realleges the allegations of the preceding paragraphs as if fully set forth herein.

55. Teva's submission of its ANDA and accompanying Paragraph IV Certifications to engage in the commercial manufacture, use, offer for sale, sale, or importation into the United States of Teva's Proposed Products, prior to the expiration of the '526 patent, constitutes infringement of one or more of the claims of that patent under 35 U.S.C. § 271(e)(2)(A).

56. A justiciable controversy exists between the parties hereto as to the infringement of the '526 patent.

57. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will infringe one or more claims of the '526 patent under 35 U.S.C. § 271(a) by making, using, offering to sell, selling, and/or importing Teva's Proposed Products in the United States.

58. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will induce infringement of one or more claims of the '526 patent under 35 U.S.C. § 271(b) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, upon FDA approval of Teva's ANDA, Teva will intentionally encourage acts of direct infringement with knowledge of the '526 patent and knowledge that its

acts are encouraging infringement. For example, according to Teva's 2nd Notice Letter, Teva will instruct prescribers to increase the dose of Teva's Proposed Products "based on a clinical assessment of tolerability and degree of improvement in Cushing's syndrome manifestations." On information and belief, Teva will also instruct prescribers that, in the absence of improvements in Cushing's syndrome manifestations, prescribers should consider measuring a trough plasma KORLYM level to guide additional titration. On information and belief, Teva will further instruct prescribers that in a study of patients with Cushing's syndrome, all patients who reached a trough KORLYM concentration of at least 2200 ng/ml had significant clinical improvement based on a centrally-adjudicated, eight-category assessment of clinical response. These instructions will cause the prescribers to perform the steps claimed in the '526 patent.

59. Unless enjoined by this Court, upon FDA approval of Teva's ANDA, Teva will contributorily infringe one or more claims of the '526 patent under 35 U.S.C. § 271(c) by making, using, offering to sell, selling, and/or importing Teva's Proposed Product in the United States. On information and belief, Teva knew and knows that Teva's Proposed Product is designed for a use that infringes one or more claims of the '526 patent, and Teva's Proposed Product lacks a substantial non-infringing use. For example, according to Teva's 2nd Notice Letter, "FDA has received a Patent Amendment to an Abbreviated New Drug Application ('ANDA') from Teva for Mifepristone Tablets, 300 mg.... The ANDA was submitted under 21 U.S.C. § 355(j)(1) and (2)(A), and contains Paragraph IV certifications to obtain approval to engage in the commercial manufacture, use or sale of Mifepristone Tablets, 300 mg, before the expiration of U.S. Patent No. 9,943,526...."

60. Failure to enjoin Teva's infringement of the '526 patent will substantially and irreparably damage Corcept.

61. Corcept does not have an adequate remedy at law.

PRAYER FOR RELIEF

62. WHEREFORE, Plaintiff Corcept respectfully requests the following relief:

(A) A Judgment that Teva infringed the '348, '495, and '526 patents by submitting ANDA No. 211436;

(B) A Judgment that Teva has infringed, and that Teva's making, using, offering to sell, selling, or importing Teva's Proposed Product will infringe one or more claims of the '348, '495, and '526 patents;

(C) An Order that the effective date of FDA approval of ANDA No. 211436 be a date no earlier than the later of the expiration of the '348, '495, and '526 patents, or any later expiration of exclusivity to which Corcept is or becomes entitled;

(D) Preliminary and permanent injunctions enjoining Teva and its officers, agents, attorneys and employees, and those acting in privity or concert with them, from making, using, offering to sell, selling, or importing Teva's Proposed Product until after the expiration of the '348, '495, and '526 patents, or any later expiration of exclusivity to which Corcept is or becomes entitled;

(E) A permanent injunction, pursuant to 35 U.S.C. § 271(e)(4)(B), restraining and enjoining Teva, its officers, agents, attorneys and employees, and those acting in privity or concert with them, from practicing any method claimed in the '348, '495, and '526 patents, or from actively inducing or contributing to the infringement of any claim of the '348, '495, and '526 patents, until after the expiration of the '348, '495, and '526 patents, or any later expiration of exclusivity to which Corcept is or becomes entitled;

(F) A Judgment that the commercial manufacture, use, importation into the United States, offer for sale, and/or sale of Teva's Proposed Product will directly infringe, induce and/or contribute to infringement of the '348, '495, and '526 patents;

(G) To the extent that Teva has committed any acts with respect the methods claimed in the '348, '495, and '526 patents, other than those acts expressly exempted by 35 U.S.C. § 271(e)(1), a Judgment awarding Corcept damages for such acts;

(H) If Teva engages in the commercial manufacture, use, importation into the United States, offer for sale, and/or sale of Teva's Proposed Product prior to the expiration of the '348, '495, and '526 patents, a Judgment awarding damages to Corcept resulting from such infringement, together with interest;

(I) A Judgment declaring that the patents-in-suit remain valid and enforceable;

(J) A Judgment awarding Corcept its costs and expenses incurred in this action; and

(K) Such further and other relief as this Court may deem just and proper.

Dated: July 6, 2018

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EXHIBIT A

US008921348B2

(12) **United States Patent**
Belanoff

(10) **Patent No.: US 8,921,348 B2**
(45) **Date of Patent: Dec. 30, 2014**

(54) **OPTIMIZING MIFEPRISTONE LEVELS IN PLASMA SERUM OF PATIENTS SUFFERING FROM MENTAL DISORDERS TREATABLE WITH GLUCOCORTICOID RECEPTOR ANTAGONISTS**

(71) Applicant: **Corcept Therapeutics**, Menlo Park, CA (US)

(72) Inventor: **Joseph K. Belanoff**, Woodside, CA (US)

(73) Assignee: **Corcept Therapeutics, Inc.**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/065,792**

(22) Filed: **Oct. 29, 2013**

(65) **Prior Publication Data**

US 2014/0162993 A1 Jun. 12, 2014

Related U.S. Application Data

(63) Continuation of application No. 12/199,114, filed on Aug. 27, 2008, now Pat. No. 8,598,149.

(60) Provisional application No. 60/969,027, filed on Aug. 30, 2007.

(51) **Int. Cl.**

A61K 31/56	(2006.01)
G01N 33/49	(2006.01)
A61K 31/575	(2006.01)

(52) **U.S. Cl.**
CPC ***G01N 33/49*** (2013.01); ***A61K 31/56*** (2013.01); ***A61K 31/575*** (2013.01)
USPC **514/178**

(58) **Field of Classification Search**
USPC 514/178
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,964,953 B2 11/2005 Belanoff

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Sarkar, "Mifepristone: bioavailability, pharmacokinetics and use-effectiveness," *European Journal of Obstetrics and Gynecology and Reproductive Biology*, vol. 101, pp. 113-120 (2002).

Primary Examiner — San-Ming Hui
(74) *Attorney, Agent, or Firm* — Kilpatrick Townsend & Stockton LLP

(57) **ABSTRACT**

The present invention provides a method for optimizing levels of mifepristone in a patient suffering from a mental disorder amenable to treatment by mifepristone. The method comprises the steps of treating the patient with seven or more daily doses of mifepristone over a period of seven or more days; testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1300 ng/mL; and adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1300 ng/mL.

7 Claims, 6 Drawing Sheets

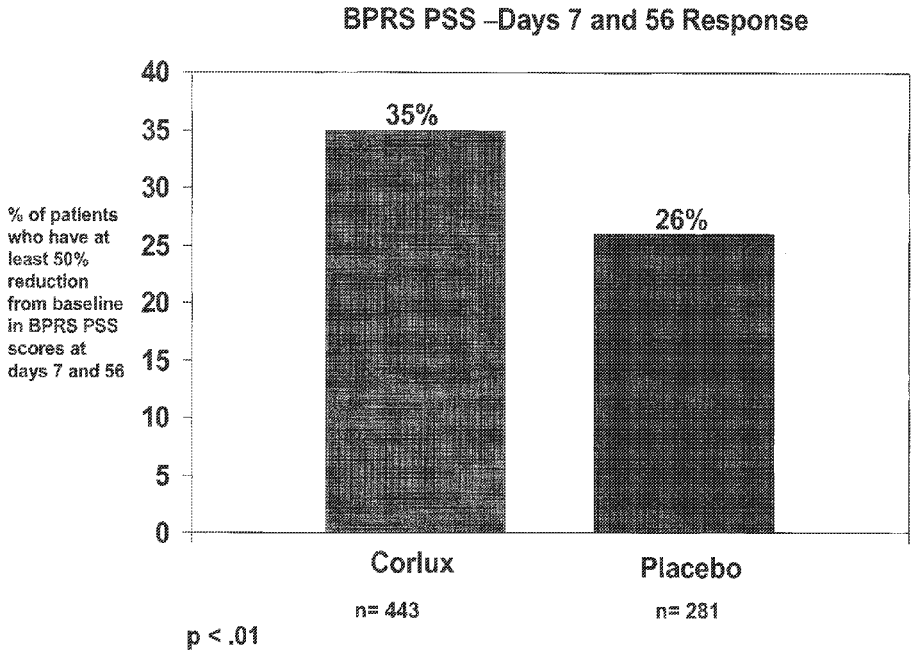


Figure 1.

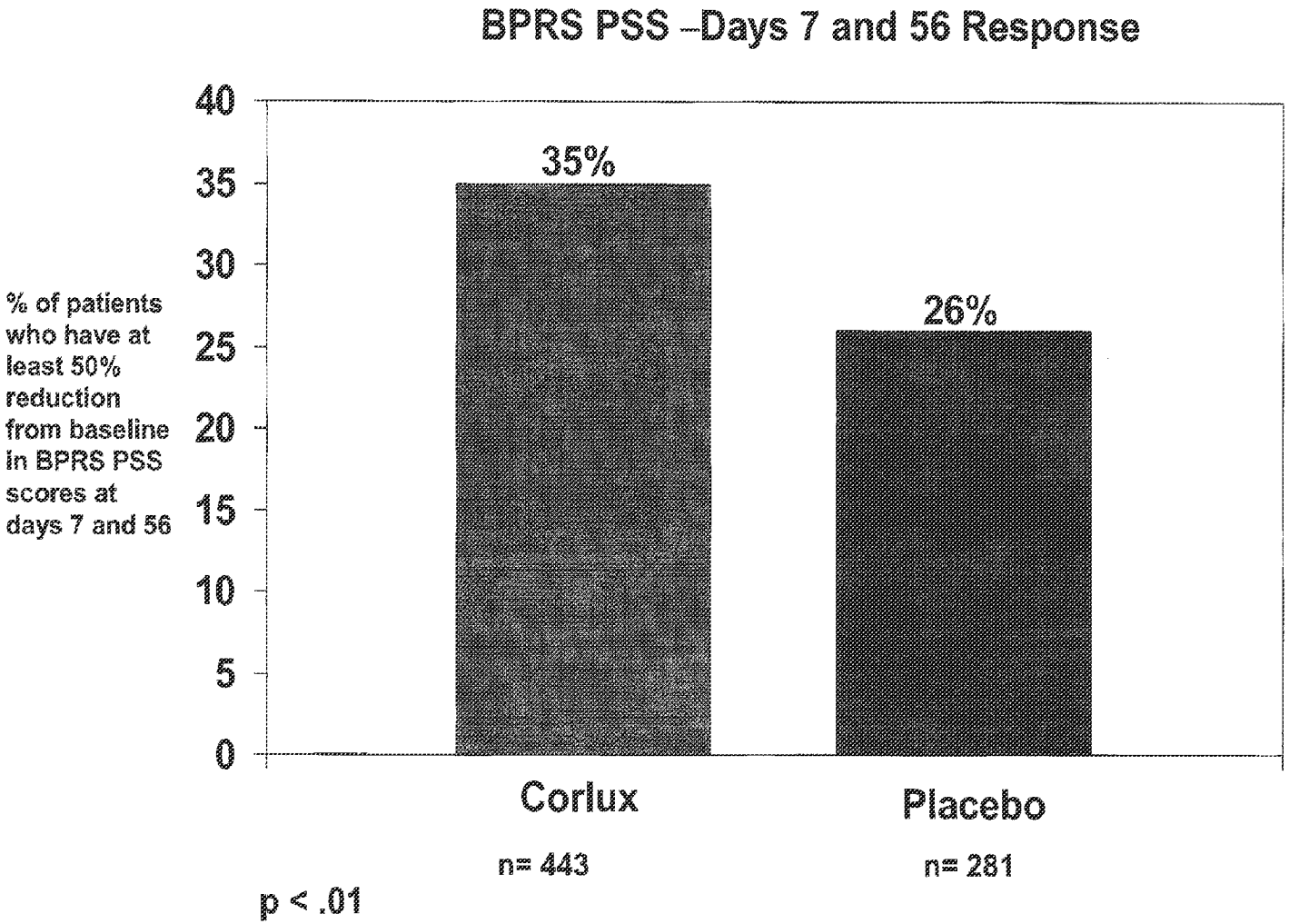

BPRS PSS –Days 7 and 56 Response
% of patients who have at least 50% reduction from baseline in BPRS PSS scores at days 7 and 56
40
35
30
25
20
15
10
5
0
35%
26%
Corlux
Placebo
n= 443
n= 281
p < .01

Figure 2.

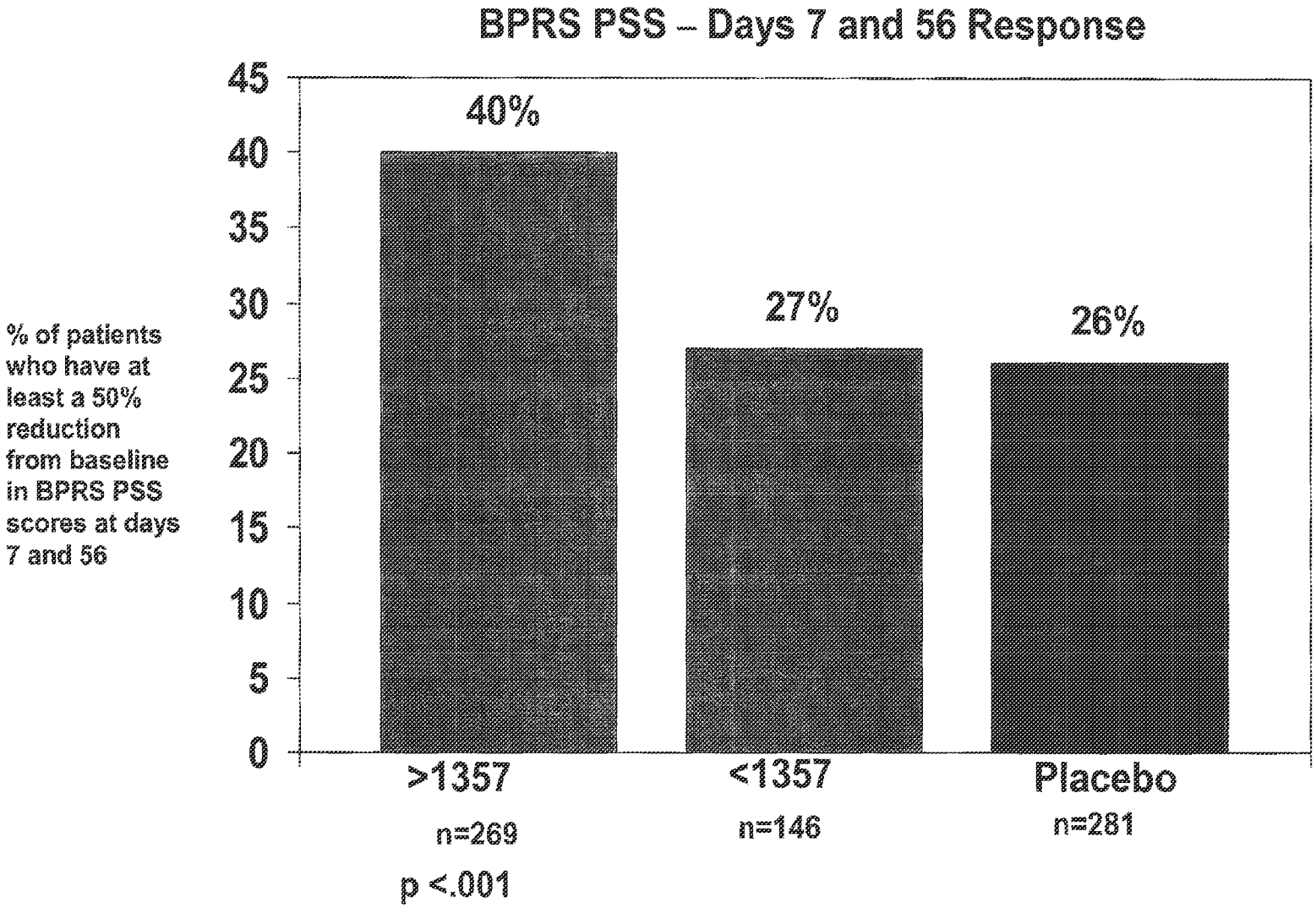

BPRS PSS – Days 7 and 56 Response
% of patients who have at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56
45
40
35
30
25
20
15
10
5
0
40%
27%
26%
>1357
n=269
<1357
n=146
Placebo
n=281
p <.001

Figure 3.

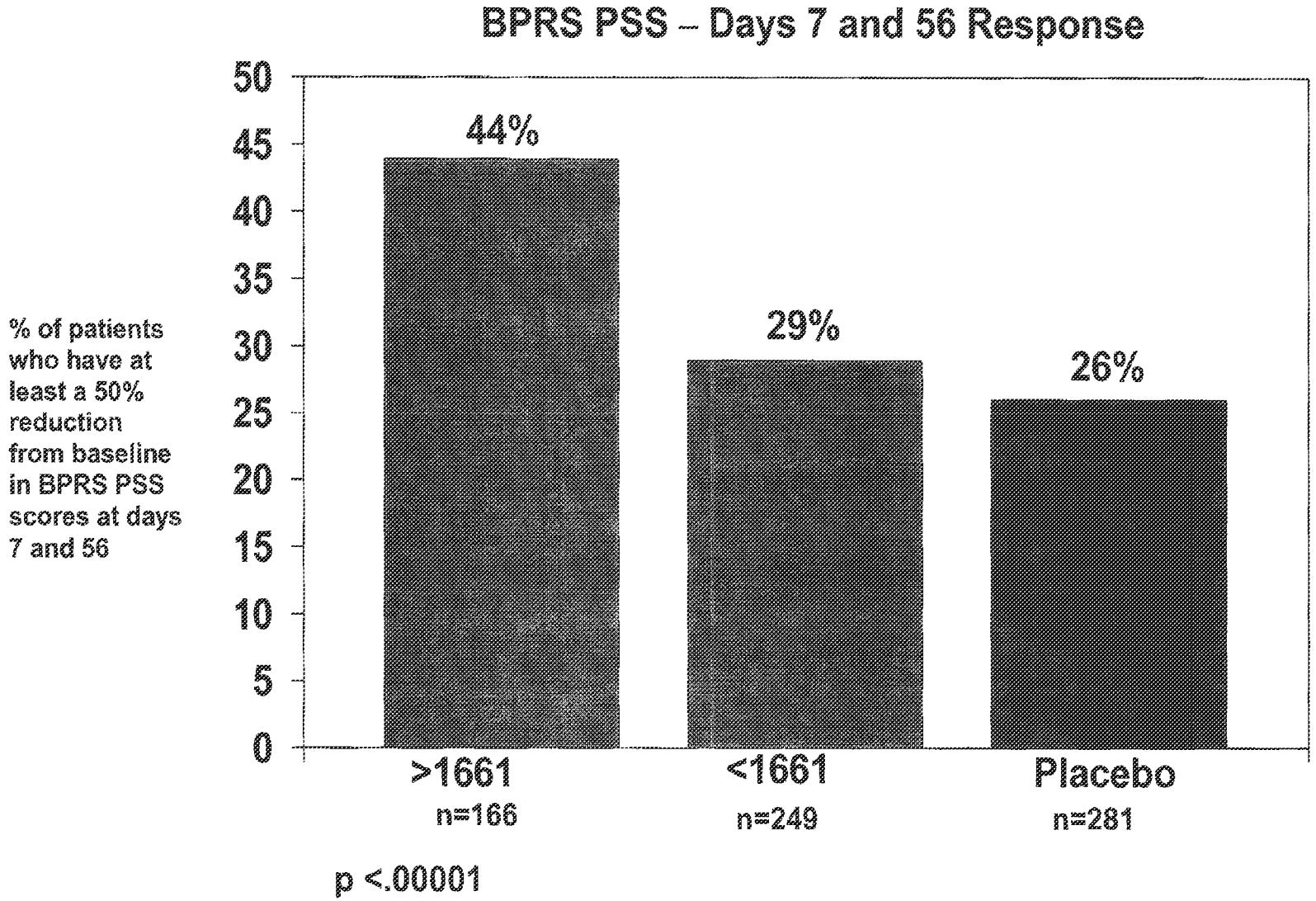

BPRS PSS – Days 7 and 56 Response
% of patients who have at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56
50
45
40
35
30
25
20
15
10
5
0
44%
29%
26%
>1661
n=166
<1661
n=249
Placebo
n=281
p <.00001

Figure 4.

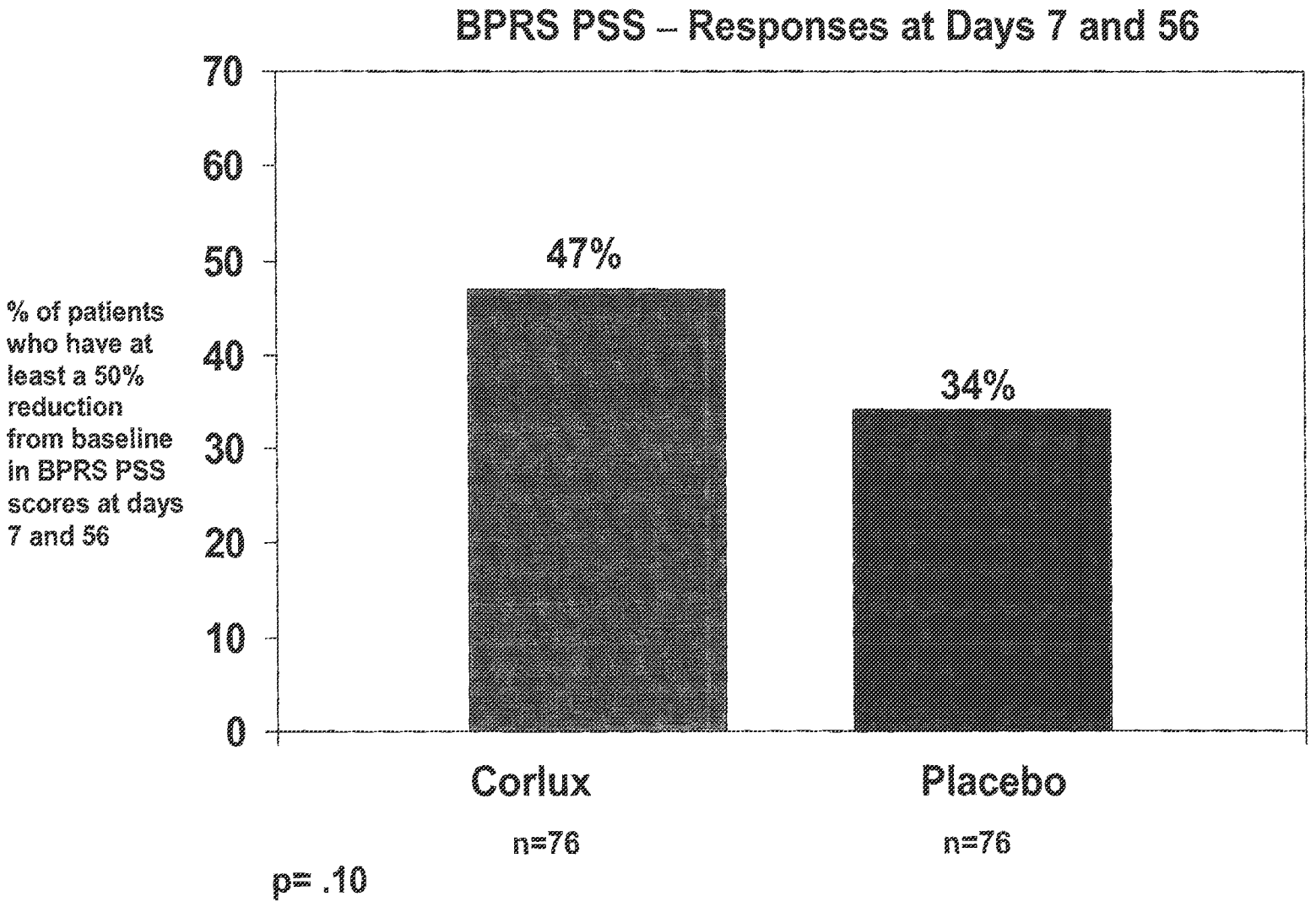

BPRS PSS – Responses at Days 7 and 56
% of patients who have at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56
70
60
50
40
30
20
10
0
47%
34%
Corlux
Placebo
n=76
n=76
p= .10

Figure 5.

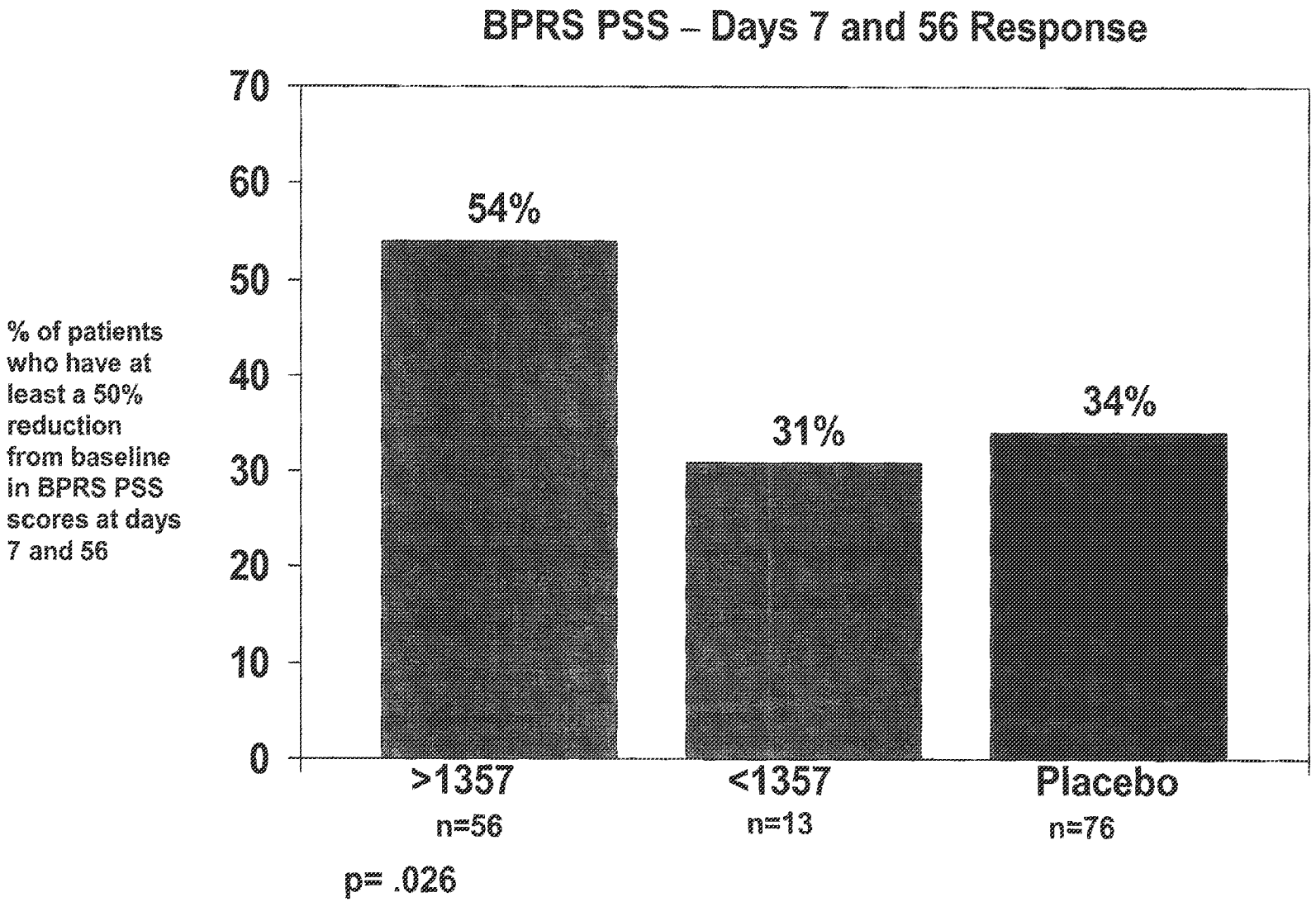
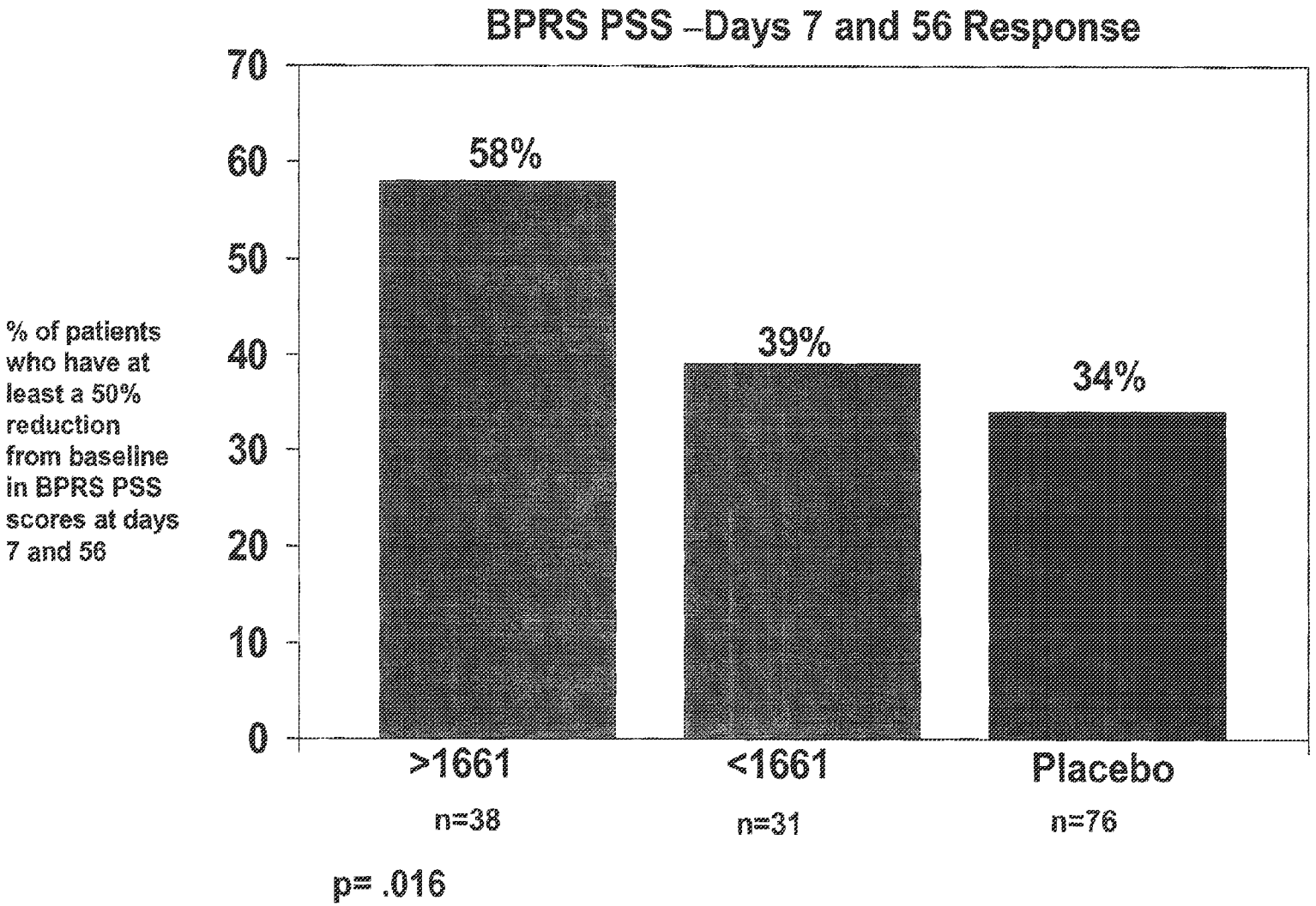

BPRS PSS – Days 7 and 56 Response
% of patients who have at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56
70
60
50
40
30
20
10
0
54%
31%
34%
>1357
n=56
<1357
n=13
Placebo
n=76
p= .026

Figure 6.


BPRS PSS --Days 7 and 56 Response
% of patients who have at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56
70
60
50
40
30
20
10
0
58%
39%
34%
>1661
<1661
Placebo
n=38
n=31
n=76
p= .016

OPTIMIZING MIFEPRISTONE LEVELS IN PLASMA SERUM OF PATIENTS SUFFERING FROM MENTAL DISORDERS TREATABLE WITH GLUCOCORTICOID RECEPTOR ANTAGONISTS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/969,027, filed Aug. 30, 2007, the disclosure of which is incorporated herein in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not Applicable

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK

Not Applicable

BACKGROUND OF THE INVENTION

It has been surprisingly discovered that administration of the same dose of mifepristone can produce widely varying blood serum levels in different patients. The varied blood serum levels can result in some patients not receiving an efficacious dose of mifepristone. For the same dose of mifepristone, the blood serum levels can differ by as much as 800% from one patient to another. Thus, a method for ensuring that the blood serum levels of mifepristone remain in an efficacious and safe range is needed.

BRIEF SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a method for optimizing levels of mifepristone in a patient suffering from a mental disorder amenable to treatment by mifepristone, the method comprising: treating the patient with seven or more daily doses of mifepristone over a period of seven or more days; testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1300 ng/mL; and adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1300 ng/mL.

In some embodiments, the mental disorder is a member selected from the group consisting of a stress disorder, delirium, mild cognitive impairment (MCI), dementia, psychosis and psychotic major depression. In other embodiments, the stress disorder is a member selected from the group consisting of Acute Stress Disorder, Post-Traumatic Stress Disorder and Brief Psychotic Disorder with Marked Stressor(s).

In another embodiment, each of the seven or more daily doses of mifepristone are administered orally. In other embodiments, the patient is treated with 28 or more daily doses over a period of 28 or more days.

In a further embodiment, the testing is performed by a plasma sampling collection device suitable for detecting mifepristone serum levels.

In other embodiments, the adjusting step comprises increasing the daily dose of the patient to achieve mifepristone blood levels greater than 1300 ng/mL.

In a second embodiment, the present invention provides a kit for treating a mental disorder amenable to treatment by mifepristone, the kit comprising: seven daily doses of mifepristone; and a plasma sampling collection device suitable for detecting mifepristone serum levels.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a comparison of patients receiving Corlux vs. placebo on primary endpoint (OC) for all studies. Of the patients receiving Corlux, 35% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 26% of patients receiving the placebo.

FIG. **2** shows a comparison of patients with plasma levels >1357 ng/mL vs. <1357 ng/mL vs. placebo (OC) for all studies. Of the patients having plasma levels of greater than 1357 ng/mL, 40% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 27% of patients having plasma levels of less than 1357 ng/mL and 26% of patients receiving the placebo.

FIG. **3** shows a comparison of patients with plasma levels >1661 ng/ml vs. placebo (OC) for all studies. Of the patients having plasma levels of greater than 1661 ng/mL, 44% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 29% of patients having plasma levels of less than 1661 ng/mL and 26% of patients receiving the placebo.

FIG. **4** shows a comparison of patients receiving Corlux vs. placebo on primary endpoint (OC) for the 1200 mg group. Of the patients receiving the 1200 mg dose of Corlux, 47% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 34% of patients receiving the placebo.

FIG. **5** shows a comparison of patients with plasma levels >1357 ng/ml vs. placebo (OC) for the 1200 mg group. Of the patients in the 1200 mg group having plasma levels of greater than 1357 ng/mL, 54% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 31% of patients having plasma levels of less than 1357 ng/mL and 34% of patients receiving the placebo.

FIG. **6** shows a comparison of patients with plasma levels >1661 ng/ml vs. placebo (OC) for the 1200 mg group. Of the patients in the 1200 mg group having plasma levels of greater than 1661 ng/mL, 58% of the patients showed at least a 50% reduction from baseline in BPRS PSS scores at days 7 and 56, versus 39% of patients having plasma levels of less than 1661 ng/mL and 34% of patients receiving the placebo.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

Administration of the same dose of mifepristone can produce widely varying mifepristone blood serum levels in different patients. For the same dose, the blood serum levels can differ by as much as 800% from one patient to another. For those patients with lower blood serum levels, the effectiveness of mifepristone treatment can suffer significantly. The present invention provides a method for optimizing the blood serum levels of mifepristone so that the blood serum levels remain in an efficacious range and the patient receives the necessary treatment.

The method of the present invention optimizes blood serum levels of mifepristone in a patient suffering from a mental disorder amenable to treatment by mifepristone by first treating the patient with mifepristone. The treatment can be for any appropriate period of time, such as seven or more daily doses over a period of seven or more days. Following

treatment for an appropriate period of time, the serum levels of the patient are tested to determine whether the blood levels of mifepristone are greater than 1300 ng/mL. The daily dose of the patient is then adjusted in order to achieve mifepristone blood levels of greater than 1300 ng/mL.

II. Definitions

The term "amenable to treatment by mifepristone" refers to a condition that is known to be treated by glucocorticoid antagonists such as mifepristone. Conditions such as mental disorders (discussed below) are amenable to treatment by mifepristone.

The term "mental disorder" refers to disorders of the mind that can be treated with a glucocorticoid antagonist such as mifepristone. Mental disorders amenable to treatment by the methods of the present invention include, but are not limited to, a stress disorder, delirium, mild cognitive impairment (MCI), dementia, psychosis and psychotic major depression.

The term "stress disorder" refers to a psychiatric condition precipitated by exposure to a traumatic or stressful event. Stress disorders include Acute Stress Disorder, Post-Traumatic Stress Disorder, and Brief Psychotic Disorder with Marked Stressor(s).

The term "Acute Stress Disorder" refers to a psychiatric condition in its broadest sense, as defined in American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, Washington, D.C., 2000 ("DSM-IV-TR"). The DSM-IV-TR defines "Acute Stress Disorder" as characterized by anxiety, dissociative, and other symptoms occurring within 1 month after exposure to an extreme traumatic stressor. The DSM-IV-TR sets forth a generally accepted standard for diagnosing and categorizing Acute Stress Disorder.

The term "Brief Psychotic Disorder with Marked Stressor(s)" refers to a psychiatric condition in its broadest sense, as defined in DSM-IV-TR. The DSM-IV-TR defines "Brief Psychotic Disorder with Marked Stressor(s)" as a sudden but brief onset of psychotic symptoms developing shortly after and apparently in response to one or more stressful events. The DSM-IV-TR sets forth a generally accepted standard for diagnosing and categorizing Brief Psychotic Disorder with Marked Stressor(s).

The term "delirium" refers to a psychiatric condition in its broadest sense, as defined in American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, Washington, D.C., 2000 ("DSM-IV-TR"). The DSM-IV-TR defines "delirium" as a disturbance of consciousness, developing over a short period of time, accompanied by a change in cognition that cannot be better accounted for by a preexisting or evolving dementia. The DSM-IV-TR sets forth a generally accepted standard for diagnosing and categorizing delirium.

The term "dementia" refers to a psychiatric condition in its broadest sense, as defined in American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Washington, D.C., 1994 ("DSM-IV"). The DSM-IV defines "dementia" as characterized by multiple cognitive deficits that include impairments in memory and lists various dementias according to presumed etiology. The DSM-IV sets forth a generally accepted standard for such diagnosing, categorizing and treating of dementia and associated psychiatric disorders.

The term "mild cognitive impairment (MCI)" refers to a category of memory and cognitive impairment that is typically characterized by a clinical dementia rating (CDR) of 0.5 (see, e.g., Hughes et al., *Brit. J. Psychiat.* 140:566-572, 1982) and further characterized by memory impairment, but not impaired function in other cognitive domains. Memory impairment is preferably measured using tests such as a "paragraph test". A patient diagnosed with MCI often exhibits impaired delayed recall performance. MCI is typically associated with aging and generally occurs in patients who are 45 years of age or older.

The term "mifepristone" refers to a family of compositions also referred to as RU486, or RU38.486, or 17-beta-hydroxy-11-beta-(4-dimethyl-aminophenyl)-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or 11-beta-(4-dimethylaminophenyl)-17-beta-hydroxy-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or analogs thereof, which bind to the glucocorticoid receptor (GR), typically with high affinity, and inhibit the biological effects initiated/mediated by the binding of any cortisol or cortisol analogue to a GR receptor (as discussed within). Salts, hydrates and prodrugs of mifepristone are all within the scope of the present invention.

The term "Post-Traumatic Stress Disorder" refers to a psychiatric condition in its broadest sense, as defined in DSM-IV-TR. The DSM-IV-TR defines "Post-Traumatic Stress Disorder" as characterized by persistent re-experiencing of an extreme traumatic event. The DSM-IV-TR sets forth a generally accepted standard for diagnosing and categorizing Post-Traumatic Stress Disorder.

The term "psychotic" as used herein refers to a psychiatric condition in its broadest sense, as defined in the DSM-IV (Kaplan, ed. (1995) supra) and described below. The term "psychotic" has historically received a number of different definitions, ranging from narrow to broadly described. A psychotic condition can include delusions or prominent hallucinations, including prominent hallucinations that the individual realizes are hallucinatory experiences, and those with hallucinations occurring in the absence of insight into their pathological nature. Finally, the term includes a psychotic condition characterized by a loss of ego boundaries or a gross impairment in reality testing. Unlike this definition, which is broad and based primarily on symptoms, characterization of psychosis in earlier classifications (e.g., DSM-II and ICD-9) were more inclusive and focused on the severity of functional impairment (so that a mental disorder was termed "psychotic" if it resulted in "impairment" that grossly interferes with the capacity to meet ordinary demands of life). Different disorders which have a psychotic component comprise different aspects of this definition of "psychotic." For example, in schizophreniform disorder, schizoaffective disorder and brief psychotic disorder, the term "psychotic" refers to delusions, any prominent hallucinations, disorganized speech, or disorganized or catatonic behavior. In psychotic disorder due to a general medical condition and in substance-induced psychotic disorder, "psychotic" refers to delusions or only those hallucinations that are not accompanied by insight. Finally, in delusional disorder and shared psychotic disorder, "psychotic" is equivalent to "delusional" (see DSM-IV, supra, page 273).

Objective tests can be also be used to determine whether an individual is psychotic and to measure and assess the success of a particular treatment schedule or regimen. For example, measuring changes in cognitive ability aids in the diagnosis and treatment assessment of the psychotic patient. Any test known in the art can be used, such as the so-called "Wallach Test," which assesses recognition memory (see below, Wallach (1980) J. Gerontol. 35:371-375). Another example of an objective text which can be used to determine whether an individual is psychotic and to measure efficacy of an antipsychotic treatment is the Stroop Color and Word Test ("Stroop Test") (see Golden, C. J., Cat. No. 30150M, In A Manual for Clinical and Experimental Uses, Stoelting, Wood Dale, Ill.) The Stroop Test is an objective neuropsychiatric

test that can differentiate between individuals with psychosis and those without, and is described in detail below.

The term "psychosis" refers to a psychiatric symptom, condition or syndrome in its broadest sense, as defined in the DSM-IV (Kaplan, ed. (1995) supra), comprising a "psychotic" component, as broadly defined above. The term psychosis can refer to a symptom associated with a general medical condition, a disease state or other condition, such as a side effect of drug abuse (a substance-induced disorder) or as a side effect of a medication. Alternatively, the term psychosis can refer to a condition or syndrome not associated with any disease state, medical condition, drug intake or the like.

Psychosis is typically defined as a mental disorder or condition causing gross distortion or disorganization of a person's mental capacity, affective response, and capacity to recognize reality, communicate, and relate to others to the degree of interfering with his capacity to cope with the ordinary demands of everyday life.

The term "psychotic major depression," also referred to as "psychotic depression" (Schatzberg (1992) Am. J. Psychiatry 149:733-745), "psychotic (delusional) depression" (Ibid.), "delusional depression" (Glassman (1981) supra) and, "major depression with psychotic features" (see the DSM-III-R), refers to a distinct psychiatric disorder which includes both depressive and psychotic features. Individuals manifesting both depression and psychosis, i.e. psychotic depression, are herein referred to as "psychotic depressives." It has been long-recognized in the art as a distinct syndrome, as described, for example, by Schatzberg (1992) supra. Illustrative of this distinctness are studies which have found significant differences between patients with psychotic and nonpsychotic depression in glucocorticoid activity, dopamine-beta-hydroxylase activity, levels of dopamine and serotonin metabolites, sleep measures and ventricle to brain ratios. Psychotic depressives respond very differently to treatment compared to individuals with other forms of depression, such as "non-psychotic major depression." Psychotic depressives have a low placebo response rate and respond poorly to antidepressant therapy alone (without concurrent anti-psychotic treatment). Psychotic depressives are markedly unresponsive to tricyclic (anti-depressive) drug therapy (Glassman, et al. (1975) supra). While psychotic depressives can respond to electroconvulsive therapy (ECT), their response time is relatively slow and the ECT has a high level of related morbidity. Clinical manifestations and diagnostic parameters of "psychotic major depression" is described in detail in the DSM-IV (Kaplan, ed. (1995) supra). Thus, due to its unique pathophysiology, high rate of morbidity and response to treatment, there is great practical need to differentially diagnose and specifically treat psychotic major depression as compared to non-psychotic depression.

The term "optimizing" refers to the process of testing mifepristone blood levels and adjusting the dosage of mifepristone administered to the patient in need in order to achieve mifepristone blood levels above 1300 ng/mL.

The terms "treat", "treating" and "treatment" collectively refer to any indicia of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being; or, in some situations, preventing the onset of dementia. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation.

The term "testing" refers to determining the mifepristone blood levels in a patient. The testing can be performed by any suitable instrument, such as a plasma sampling collection device capable of detecting mifepristone serum levels.

III. Method for Optimizing Mifepristone Levels

Administration of the same dose of mifepristone to different patients can produce widely varying blood serum levels, varying by up to 800% from one patient to another, resulting in decreased efficacy. The present invention provides a method for optimizing the blood serum levels of mifepristone so that the blood serum levels remain in an efficacious range and the patient receives the necessary treatment.

A. Patients in Need

Patients amenable to treatment with mifepristone according to the method of the present invention suffer from any mental disorder. Exemplary mental disorders include, but are not limited to, a stress disorder, delirium, mild cognitive impairment (MCI), dementia, psychosis and psychotic major depression.

Stress disorders treatable by the methods of the present invention include, but are not limited to, Acute Stress Disorder (ASD), Post-Traumatic Stress Disorder and Brief Psychotic Disorder with Marked Stressor(s).

Acute Stress Disorder (ASD) is characterized by a constellation of symptoms, lasting at least two days, that appear and resolve within one month of exposure to an extreme traumatic stressor. If symptoms appear or persist beyond one month after exposure to the traumatic stressor, the patient may be considered to suffer from Post-Traumatic Stress Disorder rather than ASD. ASD is a common precursor to Post-Traumatic Stress Disorder, and up to 80% of trauma survivors initially suffering from ASD will meet the diagnostic criteria for Post-Traumatic Stress Disorder six months later (see Brewin et al., Am J Psychiatry 156:360-6, 1999).

Patients develop ASD following exposure to an extreme traumatic stressor (DSM-IV-TR Criterion A). A person must respond to the stressor with intense fear, helplessness, or horror to be diagnosed with ASD. ASD may develop from direct experience of traumatic events, including violent crimes, physical trauma, combat, diagnosis with a life-threatening illness, and natural or manmade disasters. Patients may also develop ASD from witnessing or learning about traumatic events that happen to others, especially family members or close friends. Unexpected exposure to death, dead bodies, or body parts may also induce ASD.

A diagnosis of ASD requires that the person meet several other symptomatic criteria. The person must experience three or more dissociative symptoms in connection with the traumatic stressor (Criterion B). Dissociative symptoms include a subjective sense of numbing or detachment, a reduction in awareness of surroundings, derealization, depersonalization, and dissociative amnesia. Furthermore, ASD requires that the victim persistently re-experience the traumatic event, though recurrent images, thoughts, dreams, illusions, flashbacks, sense of reliving the event, or distress upon exposure to reminders of the event (Criterion C). The person must display marked avoidance of stimuli that arouse recollection of the trauma (Criterion D) and marked symptoms of anxiety or increased arousal (Criterion E). Finally, in addition to the time requirements described above, a diagnosis of ASD requires that the disturbance cause significant distress; or life impairment, and not be due to another psychiatric or physiological condition (Criteria F-H).

Like Acute Stress Disorder, Post-Traumatic Stress Disorder (PTSD) emerges following exposure to an extreme trau-

matic stressor, and is characterized by persistent reexperiencing of the traumatic event, avoidance of stimuli associated with the trauma, and anxiety or increased arousal. The types of traumatic stressors giving rise to PTSD, and the manifestations of PTSD symptoms, are identical to those described above for ASD, but for three differences. First, the dissociative symptoms required for a diagnosis of ASD are not required for a diagnosis of PTSD, although dissociative symptoms may commonly be seen in PTSD patients. Secondly, PTSD need not arise within one month of exposure to the traumatic stressor, and may emerge months or years after the traumatic event. Thirdly, in contrast to the one month maximum duration of symptoms required for a diagnosis of ASD, symptoms must persist for at least one month in order for a diagnosis of PTSD to be made.

A Brief Psychotic Disorder is a short-term (between one day and one month) disturbance involving the sudden onset of at least one psychotic symptom, such as delusions, hallucinations, disorganized speech, or grossly disorganized or catatonic behavior. Brief Psychotic Disorders exclude those induced by a general medical condition. If psychotic symptoms develop shortly after, and apparently in response to, one or more severely stressful events, the disturbance is diagnosed as Brief Psychotic Disorder with Marked Stressor(s) (formerly labeled "brief reactive psychosis" in DSM-III-R). Brief Psychotic Disorder with Marked Stressor(s) is treatable by the glucocorticoid receptor antagonists of the present invention.

Delirium is characterized by disturbances of consciousness and changes in cognition that develop over a relatively short period of time. The disturbance in consciousness is often manifested by a reduced clarity of awareness of the environment. The patient displays reduced ability to focus, sustain or shift attention (DSM-IV-TR diagnostic Criterion A). Accompanying the disturbance in consciousness, delirium patients display a disturbance in cognition (e.g., memory impairment, disorientation, language difficulties) or perceptual disturbances (e.g., misinterpretations, illusions, or hallucinations) (Criterion B). To be considered delirium, these disturbances in consciousness, cognition, or perception should develop over a short period of time and tend to fluctuate during the course of the day (Criterion C).

Delirium may arise from a number of general medical conditions, including central nervous system disorders (e.g., trauma, stroke, encephalopathies), metabolic disorders (e.g., renal or hepatic insufficiency, fluid or electrolyte imbalances), cardiopulmonary disorders (e.g., congestive heart failure, myocardial infarction, shock), and systemic illnesses or effects (e.g., infections, sensory deprivation, and postoperative states). Glucocorticoid receptor antagonists are also effective to treat Substance-Induced Delirium (e.g., delirium induced by substance intoxication or withdrawal, medication side effects, and toxin exposure). Delirium may arise from multiple simultaneous etiologies (e.g., a combination of a general medical condition and substance intoxication) and such delirium, as well as delirium of unknown or unclassified origin, may be treated with the glucocorticoid receptor antagonists of the present invention.

Mild cognitive impairment (MCI) is characterized as a mild impairment of cognition categorized as a CDR of 0.5 that is associated with deficits in a memory task test, such as a paragraph test. An MCI patient is fully oriented, but demonstrates mild consistent forgetfulness. Impairment in cognitive domains other than memory, such as problem solving and judgment is doubtful, if present at all, and, further, the MCI patient does not demonstrate impairment in functioning in the community or at home. A patient with MCI scores normally on standard tests of dementia.

There are various means to diagnose the onset of MCI and to assess the efficacy of treatment using the methods of the invention. These include the administration of psychiatric tests to determine the CDR, the administration of memory tests, and the administration of psychiatric tests for dementia, which are used to exclude a diagnosis of dementia. The results of these test may be considered in conjunction with other objective physical tests as described below. These means are also useful for assessing the efficacy of the methods of the invention in improving memory or decreasing or diminishing further impairment in memory or cognitive decline in a patient with MCI. Subjective and objective criteria can be used to measure and assess the success of a particular GR antagonist, pharmaceutical formulation, dosage, treatment schedule or regimen. The features (symptoms) of and criteria for diagnosing MCI are described, e.g., in Petersen et al., Arch. Neurol. 56:303-308, 1999.

The dementia treated in the methods of the invention encompasses a broad range of mental conditions and symptoms, as broadly described in the DSM-IV. While the practitioner can use any set of prescribed or empirical criteria to diagnose the presence of dementia as an indication to practice the methods of the invention, some illustrative diagnostic guidelines and examples of relevant symptoms and conditions are described below.

The DSM-IV states that dementias typically associated with Alzheimer's disease (dementia of the Alzheimer's type), "vascular dementia" (also known as multi-infarct dementia), or "dementia due to general medical conditions," e.g., human immunodeficiency virus (HIV-1) disease, head trauma, Parkinson's disease, or Huntington's disease (further discussed, below). Dementias can also be "substance-induced persisting dementia," i.e., due to a drug of abuse, a medication, or toxin exposure, "dementia due to multiple etiologies," or a "dementia not otherwise specified" if the etiology is indeterminate.

Psychosis can be manifested as a mental illness in the form of a syndrome or as an element of a variety of disease processes. There are various means to diagnose these various forms of psychosis and assess the success of treatment. These means include classical psychological evaluations in addition to the various laboratory procedures described above. Such means are well-described in the scientific and patent literature, and some illustrative examples are provided below.

The psychosis ameliorated in the methods of the invention encompasses a broad range of mental conditions and symptoms, as broadly described in the DSM-IV (Kaplan, ed. (1995) supra). Psychosis can refer to a symptom associated with a general medical condition, a disease state or other condition, such as a side effect of drug abuse (a substance-induced disorder) or as a side effect of a medication. While the practitioner can use any set of proscribed or empirical criteria to diagnose the presence of a psychosis as an indication to practice the methods of the invention, some illustrative diagnostic guidelines and examples of relevant symptoms and conditions are described below.

Psychiatric conditions, such as psychosis, can be further diagnosed and evaluated using any of the many tests or criteria well-known and accepted in the fields of psychology or psychiatry.

The features (symptoms) of and criteria for diagnosing psychotic disorders, such as psychotic major depression, are further described DSM-IV, supra. While the practitioner can use any criteria or means to evaluate whether an individual is psychotic to practice the methods of the invention, the DSM-

IV sets forth a generally accepted standard for such diagnosing, categorizing and treating of psychiatric disorders, including psychosis. Several illustrative examples of such criteria utilized in the methods of the invention are set forth below.

Psychosis is typically characterized as a mental disorder or condition causing gross distortion or disorganization of a person's mental capacity, affective response, and capacity to recognize reality, communicate, and relate to others to the degree of interfering with his capacity to cope with the ordinary demands of everyday life. In a condition or illness involving psychosis, delusions or hallucinations can be present. The content of the delusions or hallucinations have many depressive themes. In psychotic major depression there can be "mood-congruent" psychotic features, including, for example, delusions of guilt, delusions one deserves punishment (e.g. because of a personal inadequacy or moral transgression), nihilistic delusions (e.g. of world or personal destruction), somatic delusions (e.g. having cancer), or delusions of poverty. Hallucinations, when present in psychotic major depression are usually transient and not elaborate and may involve voices that berate the patient for shortcomings or sins. More rarely, the content of the delusions or hallucinations has no apparent relationship to depressive themes. In this situation these "mood-incongruent" psychotic features include, for example, grandiose delusions.

Psychosis can also include bipolar I disorder with psychotic features, brief psychotic disorder, delusional disorder, shared psychotic disorder, substance induced psychotic disorder and psychotic disorder not otherwise specified.

B. Formulations of Mifepristone

Formulations of the present invention include mifepristone in combination with pharmaceutical excipients. Mifepristone is commercially available from a variety of sources such as Eurolabs Ltd. (London, England). Mifepristone can also be synthesized by one of skill in the art using known synthetic procedures.

The term "mifepristone" refers to a family of compositions also referred to as RU486, or RU38.486, or 17-beta-hydroxy-11-beta-(4-dimethyl-aminophenyl)-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or 11-beta-(4-dimethylaminophenyl)-17-beta-hydroxy-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or analogs thereof, which bind to the GR, typically with high affinity, and inhibit the biological effects initiated/mediated by the binding of any cortisol or cortisol analogue to a GR receptor. Chemical names for RU-486 vary; for example, RU486 has also been termed: 11B-[p-(Dimethylamino)phenyl]-17B-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one; 11B-(4-dimethyl-aminophenyl)-17B-hydroxy-17A-(prop -1-ynyl)-estra-4,9-dien-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A -(propynyl-1)-estra-4,9-diene-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A -(propynyl-1)-E; (11B,17B)-11-[4-dimethylamino)-phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one; and 11B-[4-(N,N-dimethylamino) phenyl]-17A-(prop-1-ynyl)-D-4,9-estradiene-17B-ol-3-one. Salts, hydrates and prodrug forms of mifepristone are also useful in the formulations of the present invention.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of mifepristone suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents. Preferred pharmaceutical preparations can deliver the compounds of the invention in a sustained release formulation.

C. Administration of Mifepristone

The formulations of the present invention provide serum levels of mifepristone of at least 1300 ng/mL. The mifepristone utilized in the pharmaceutical method of the invention is administered at the initial dosage of about 0.001 mg/kg to about 1000 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the patient and the condition being treated. The dose administered to a patient, in the context of the present invention, should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound in a particular patient. Determination of the proper dosage for a particular situation is within the skill of the practitioner.

Generally, treatment is initiated with six daily doses, with the blood levels tested on the day of the seventh daily dose in order to determine whether the dose used is providing a mifepristone blood level of at least 1300 ng/mL. The testing is also performed to ensure the blood levels are below those afforded by an LD50 dose of about 1000 mg/kg. If the mifepristone blood level is lower than 1300 ng/mL. Additional testing of mifepristone blood levels can be necessary in order to confirm a mifepristone blood level of at least 1300 ng/mL or to adjust the mifepristone daily dose higher. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired. In addition, the interval from initiation of treatment and testing for mifepristone blood levels can be as short as 1 daily dose, or up to 28 daily doses and longer.

Mifepristone can be administered for any period of time, such as 7 daily doses over a period of seven days. Mifepristone can also be administered using more daily doses over a longer period of time, such as via 28 daily doses over a period of 28 days. Longer times for administration of mifepristone are also within the scope of the present invention.

D. Assay for Testing Mifepristone Levels

Mifepristone levels can be determined by any method known in the art. Methods for detecting mifepristone levels include, but are not limited to, radio-immuno assay and mass spectrometry (MALDI, SELDI, LS/MS, LS/MS/MS, among others). Liquid chromatography mass spectrometry (LC/MS

or LC-MS) separates compounds chromatographically before they are introduced to the ion source and mass spectrometer. It differs from GC/MS in that the mobile phase is liquid, usually a combination of water and organic solvents, instead of gas. Most commonly, an electrospray ionization source is used in LC/MS.

Tandem mass spectrometry (MS/MS) involves multiple steps of mass selection or analysis, usually separated by some form of fragmentation. A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry. For example, one mass analyzer can isolate one peptide from many entering a mass spectrometer. A second mass analyzer then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A third mass analyzer then catalogs the fragments produced from the peptides. Tandem MS can also be done in a single mass analyzer over time as in a quadrupole ion trap. There are various methods for fragmenting molecules for tandem MS, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD) and blackbody infrared radiative dissociation (BIRD). One of skill in the art will appreciate that other assays for testing mifepristone levels are known to one of skill in the art.

In some embodiments, the assay can be performed as follows. Blood is collected from a patient in a vacutainer containing sodium heparin. The blood is centrifuged and the resulting plasma frozen at an appropriate temperature until assay. In some embodiments, the temperature is about −70° C. In other embodiments, other blood components can be collected and stored. Prior to analysis, the plasma is thawed and a fraction of the plasma is mixed with an internal standard in a solvent such as acetonitrile, to obtain a fixed concentration of the standard. In some embodiments, the internal standard can be mifepristone-d_4. The concentration of the internal standard is selected in order to be greater than the expected concentration of mifepristone in the plasma. For example, the internal standard can have a concentration of 2000 ng/mL. One of skill in the art will appreciate that other internal standards, and other concentrations, are useful in the present invention.

Base is then added to the sample solution. The base can be any amine or ammonium base, such as ammonium hydroxide. One of skill in the art will appreciate that other bases are useful in the present invention.

Solvent is then added to the solution and the mifepristone (along with the internal standard) are extracted from the plasma. Solvents useful for the extraction of mifepristone include, but are not limited to, hexanes, pentanes, ethers (such as diethylether, tetrahydrofuran and methyl-t-butyl ether (MTBE)), ethyl acetate, chloroform and methylene chloride. One of skill in the art will appreciate that other solvents are useful in the present invention.

Following separation and concentration of the organic layer, the sample is reconstituted in a solvent mixture comprising water, acetonitrile and formic acid. The ratio of the solvent components can vary. In some embodiments, the solvent mixture is water:acetonitrile:formic acid (75:25:0.1, v/v/v). One of skill in the art will appreciate that other solvent mixtures are useful in the present invention.

The sample can then be analyzed by reverse-phase high pressure liquid chromatography (HPLC). In some embodiments, the reverse-phase HPLC is performed using a water:acetonitrile:formic acid (60:40:0.1) mobile phase (isocratic) at a flow rate of 0.3 mL/min. One of skill in the art will appreciate that other mobile phases and flow rates are useful in the present invention.

The reverse-phase HPLC column can be a phenyl column maintained at 50° C. Mifepristone elutes at 4.2 minutes. Following elution, the mobile phase can be nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds detected using a tandem quadrupole mass spectrometer. Mifepristone (molecular weight of 430 g/mol) can be detected at m/z 372.30. The internal standard mifepristone-d_4 can be detected at m/z 376.30. The ratio of the mifepristone peak height to the peak height for the internal standard can then be calculated.

The plasma concentration of mifepristone is then calculated by comparing the experimental ratio to a standard curve of mifepristone:mifepristone-d_4 peak height ratio v. mifepristone concentration. The standard curve is generated by first measuring the mifepristone:mifepristone-d_4 peak height ratios for mifepristone samples at 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL where the mifepristone-d_4 internal standard has a concentration of 2000 ng/mL. The mifepristone:mifepristone-d_4 peak height ratios of these known solutions are then fit to a power equation (Mass Lynx by Micromass, Beverly, Mass.), against which future samples with unknown concentrations of mifepristone are compared.

The plasma levels of mifepristone derivatives such as RU42633, RU42698 and RU42848, among others, can also be determined using the assay described above.

E. Kits for Treating Mental Disorders with Mifepristone

The present invention provides kits. The kits of the present invention comprise seven daily doses and a plasma sampling collection device. The kits of the present invention can also comprise any other component necessary for a kit, such as a container.

Patient plasma can be collected by any known plasma collection device. Some plasma collection devices useful in the present invention include, but are not limited to, vacutainers. The plasma collection devices of the present invention can optionally comprise additives in the device, such as anticoagulants (EDTA, sodium citrate, heparin, oxalate), a gel with intermediate density between blood cells and blood plasma, particles causing the blood to clot, a gel to separate blood cells from serum, thrombin and fluoride, among others.

The kits can also contain additional vessels used for further analysis of the plasma. For example, when the plasma is centrifuged, the centrifuged plasma can be transferred to a vessel, such as a cryostat tube. One of skill in the art will appreciate that other vessels and containers are useful in the present invention.

IV. EXAMPLES

Example 1

Determination of Mifepristone Plasma Level

This example provides a procedure for determining the plasma level of mifepristone in a patient.

Three (3) mL of blood was collected from a patient in a vacutainer containing sodium heparin. The blood was centrifuged and the resulting plasma frozen at −70 to −80° C. until assay. For analysis, the plasma samples were warmed and prepared as follows:

1. Using a pipette, 50.0 μL of the sample was aliquoted into a 16×100-mm glass test tube. When a partial volume aliquot was needed, the aliquot was added to the tube and diluted to full volume with blank human plasma.
2. 20.0 μL of the internal standard, mifepristone-d_4 (5.00 μg/mL in acetonitrile), was added to the tube, resulting in 2000.0 ng/mL mifepristone-d_4 in plasma.

3. The tube was vortexed for approximately 1 minute.
4. 50.0 μL of 6% ammonium hydroxide was added to the tube.
5. The tube was vortexed for approximately 1 minute.
6. 2.00 mL of MTBE was added to the tube.
7. 2.00 mL of hexane was added to the tube.
8. The tube was vortexed for at least 15 minutes.
9. The tube was centrifuged for at least 10 minutes at 2500 RPM (575×g).
10. The aqueous layer was frozen in a freezer set to maintain −70° C.
11. The upper organic layer was poured into a 13×100-mm polypropylene tube.
12. The organic layer was evaporated in a Turbovap set to 40° C.
13. 200.0 μL of a solution of water:acetonitrile:formic acid (75:25:0.1, v/v/v) was added to the tube.
14. The tube was vortexed for approximately 1 minute.
15. The tube was sonicated for approximately 1 minute.
16. The tube was vortexed for approximately 1 minute.
17. The sample was transferred to a labeled injection vial or well plate.
18. The vial or plate was capped and checked for air bubbles.

The sample was then analyzed by reverse-phase high pressure liquid chromatography using a water:acetonitrile:formic acid (60:40:0.1) mobile phase (isocratic) at a flow rate of 0.3 mL/min. The column was a phenyl column maintained at 50° C. Mifepristone elutes at 4.2 minutes. Following elution, the mobile phase was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds detected using a tandem quadrupole mass spectrometer. Mifepristone (molecular weight of 430 g/mol) was detected at m/z 372.30. The internal standard mifepristone-d_4 was detected at m/z 376.30. The ratio of the mifepristone peak height to the mifepristone-d_4 peak height was calculated.

The plasma concentration of mifepristone was then calculated by comparing the experimental ratio to a standard curve of mifepristone:mifepristone-d_4 peak height ratio v. mifepristone concentration. The standard curve was generated by first measuring the mifepristone:mifepristone-d_4 peak height ratios for mifepristone samples at 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL where the mifepristone-d_4 internal standard has a concentration of 2000 ng/mL. The mifepristone:mifepristone-d_4 peak height ratios of these known solutions were then fit to a power equation (Mass Lynx by Micromass, Beverly, Mass.), and the sample with unknown concentrations of mifepristone was compared.

Example 2

Phase III Trial with Three Dose Levels of CORLUX™

This example provides a randomized, double-blind, placebo-controlled, parallel group study of the safety and efficacy of three dose levels of CORLUX™ (Mifepristone) plus an antidepressant vs. placebo plus an antidepressant in the treatment of psychotic symptoms in patients with major depressive disorder with psychotic features (PMD).

The study was a Phase III trial performed using several investigators at several different sites. The objectives were to demonstrate the efficacy and safety of three dose levels of CORLUX (mifepristone) combined with an antidepressant compared to placebo combined with an antidepressant in the treatment of psychotic symptoms in patients with Major Depressive Disorder with Psychotic Features (PMD).

The number of patients was less than 440. Patients eligible for randomization were male or nonpregnant female outpatients, and inpatients, if clinically required, with a diagnosis of Major Depressive Disorder with Psychotic Features (DSM-IV 296.24 or 296.34), and a BPRS Positive Symptom subscale score of at least 12, a BPRS total score of at least 38, and a HAMD-24 score of at least 20.

CORLUX was used as the test drug at 300 (1×300 mg tablet), 600 (2×300 mg tablet), and 1200 mg (4×300 mg tablet) once a day by mouth for the initial 7 days. Appropriate numbers of active and placebo tablets will be given to all dose groups so that each patient takes a total of 4 tablets at each daily dose. The reference drug was a placebo (1, 2, or 4 tablets matching CORLUX 300 mg tablets) once a day by mouth for the initial 7 days.

Up to 440 patients were randomly assigned to receive CORLUX 300, 600, or 1200 mg/day or placebo (in a 1:1:1:1 ratio) each day for 7 days. An antidepressant selected from a prescribed list was started simultaneously with the study drug, and continued to the end of the trial. BPRS and HAM-D assessments were performed at Screen and on Days 0, 7, 14, 28, 42, and 56, and at early termination when it occurred. Safety visits occurred at Days 21 and 35. The patients who are seen as outpatients made daily visits to the clinic setting to receive study medications for the first 7 days. If clinically necessary, a patient was treated as an inpatient.

In addition to the selected antidepressant, continuing benzodiazepines was allowed up to specified dose levels, but antipsychotics, mood stabilizers and additional antidepressants were not allowed during the entire study. If the patient was at imminent risk to him/herself and/or others and therefore could not be adequately treated within the study (e.g., required ECT, new or re-hospitalization for PMD, antipsychotics or mood stabilizers, or a second antidepressant), the patient underwent an early termination visit on the day that rescue therapy was started and completed final efficacy evaluations. If early termination occurred prior to day 35, the patient returned for a safety follow up visit at day 35.

The primary efficacy endpoint was the proportion of patients with at least a 50% reduction from baseline of the BPRS Positive Symptom Subscale (PSS) scores at Days 7 and 56. The secondary endpoints were: (1) the proportion of responders at days 7 and 28; and (2) the mean change from baseline to day 56 in the HAM-D-24 total score.

Adverse events, laboratory assessments including electrocardiograms, and physical examination were used to assess safety.

The criteria for assessing study efficacy objective was the proportion of patients with a reduction of at least 50% from baseline in BPRS Positive Symptom Subscale scores at Days 7 and 56.

Example 3

Phase III Trial for Study of the Efficacy and Safety of CORLUX™

This example provides an international, double-blind, placebo-controlled study of the efficacy and safety of CORLUX™ (Mifepristone) vs. placebo in the treatment of psychotic symptoms in patients with Psychotic Major Depression (PMD).

The study was a Phase III trial performed using several investigators at several different international sites. The objective of the trial was to demonstrate the efficacy and safety of CORLUX (mifepristone) combined with an antidepressant compared to placebo combined with an antidepres-

sant in the treatment of psychotic symptoms in patients with Major Depressive Disorder with Psychotic Features (PMD).

The number of patients was 220 evaluable subjects. Patients eligible for randomization were male or non-pregnant female outpatients, or inpatients, if necessary for patient well-being, with a diagnosis of Major Depressive Disorder with Psychotic Features (ICD-10 F32.3 or F33.3 or DSM-IV 296.24 or 296.34). At the screening and baseline visits, patients demonstrated the following severity of illness: BPRS Positive Symptom Subscale (PSS) score ≥12; BPRS total score ≥38, and HAMD-24 total score ≥20.

CORLUX was administered in a 600 mg dose once a day by mouth for the initial 7 days (administered as two 300 mg tablets). Reference drug, dose, dosage regimen, route of administration: Matching placebo was administered once a day by mouth for the initial 7 days.

Up to 280 patients were randomly assigned (1:1 ratio) to receive either CORLUX 600 mg/day or placebo daily for 7 days. After the 7-day dosing period, patients were evaluated at Days 14, 21, 28, 35, 42 and 56. An antidepressant was administered simultaneously with study drug, and continued to the end of the trial (Day 56). BPRS and HAMD-24 assessments were performed at Screen and on Days 0, 7, 14, 28, 42 and 56, or at early termination. A safety visit occurred on Days 21 and 35, and at study termination on Day 56. Subjects treated on an outpatient basis made daily visits to the clinic to receive study medication for the first 7 days. Subjects were treated on an inpatient basis for as long as deemed clinically necessary by the investigator.

In addition to the selected antidepressant, concomitant benzodiazepine treatment was allowed up to specified dose levels. Antipsychotics, mood stabilizers and a second antidepressant were prohibited during the entire study. If the patient was at imminent risk to him/herself and/or others and therefore could not be adequately treated within the study (i.e., required ECT, new or re-hospitalization for PMD, antipsychotics or mood stabilizers, or a second antidepressant), the patient underwent an early termination visit on the day that rescue therapy was started, and completed procedures listed for the day 56 termination visit, including final efficacy evaluations. If early termination occurred prior to day 35, the patient returned for a safety follow-up visit at regularly scheduled day 35.

The Primary efficacy endpoint was determined by the proportion of patients with ≥50% reduction from baseline on the BPRS-PSS at Days 7 and 28. Key secondary efficacy endpoints include (1) the proportion of patients with ≥50% reduction from baseline on the BPRS-PSS at Days 7 and 56; and (2) change from baseline on the HAMD-24 at Day 56.

Adverse events, laboratory assessments including electrocardiograms, and physical examination were used to assess safety.

Example 4

Treatment of Male Patient with PMD

A 50 year-old male, weighing 175 pounds, presents to physician with psychotic major depression (PMD). The physician prescribes 300 mg of mifepristone for seven daily doses over a period of seven days. One week later on the day of the seventh daily dose, three (3) mL of blood are collected from the patient and analyzed as described above in the specification. The dose of mifepristone is then adjusted, if necessary, to achieve mifepristone blood levels of greater than 1300 ng/mL. The mifepristone dose can be adjusted a single time to achieve mifepristone blood levels of greater than 1300 ng/mL. Alternatively, several adjustments to the mifepristone dose can be necessary to safely achieve mifepristone blood levels of greater than 1300 ng/mL.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.

What is claimed is:

1. A method for optimizing levels of mifepristone in a patient suffering from a disorder amenable to treatment by mifepristone, the method comprising:

treating the patient with seven or more daily doses of mifepristone over a period of seven or more days;

testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1300 ng/mL; and

adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1300 ng/mL.

2. The method of claim **1**, wherein the disorder is a member selected from the group consisting of a stress disorder, delirium, mild cognitive impairment (MCI), dementia, psychosis and psychotic major depression.

3. The method of claim **2**, wherein the stress disorder is a member selected from the group consisting of Acute Stress Disorder, Post-Traumatic Stress Disorder and Brief Psychotic Disorder with Marked Stressor(s).

4. The method of claim **1**, wherein each of the seven or more daily doses of mifepristone are administered orally.

5. The method of claim **1**, wherein the patient is treated with 28 or more daily doses over a period of 28 or more days.

6. The method of claim **1**, wherein the testing is performed by a plasma sampling collection device suitable for detecting mifepristone serum levels.

7. The method of claim **1**, wherein the adjusting step comprises increasing the daily dose of the patient to achieve mifepristone blood levels greater than 1300 ng/mL.

* * * * *

EXHIBIT B

US009829495B2

(12) **United States Patent**
Moraitis

(10) **Patent No.: US 9,829,495 B2**
(45) **Date of Patent: Nov. 28, 2017**

(54) **METHOD FOR DIFFERENTIALLY DIAGNOSING ACTH-DEPENDENT CUSHING'S SYNDROME**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

This invention provides for an improved method for differentially diagnosing ACTH-dependent Cushing's syndrome. Current practice for differentially diagnosing ectopic ACTH syndrome and Cushing's Disease measures relative ACTH concentrations from the inferior petrosal venous sinus compared to fluid obtained from a periphery venous sample. This is performed before and after administration of exogenous corticotropin releasing factor, or after administration of metyrapone. This invention uses glucocorticoid receptor antagonists to induce release of endogenous CRH which stimulates ACTH to increase in patients with ectopic ACTH syndrome but not in those with Cushing's Disease.

18 Claims, No Drawings

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METHOD FOR DIFFERENTIALLY DIAGNOSING ACTH-DEPENDENT CUSHING'S SYNDROME

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims benefit of U.S. provisional application No. 62/204,723, filed Aug. 13, 2015, the entire content of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Cortisol is a steroid produced by the adrenal glands and is used in the body to respond to physical and emotional stress and to maintain adequate energy supply and blood sugar levels. Cortisol production is highly regulated by the hypothalamic-pituitary-adrenal axis (HPA) through a complex set of direct influences and negative feedback interactions. In healthy individuals, insufficient cortisol in the bloodstream triggers the hypothalamus to release corticotropin-releasing hormone (CRH) which signals to the pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal glands to produce more cortisol. Excessive cortisol inhibits hypothalamus from producing CRH, thus inhibiting the pituitary gland from releasing ACTH, which in turn suppresses cortisol production. The HPA regulation also results in a diurnal rhythm of cortisol levels, reaching peaks in the morning and nadirs around midnight. Pathological conditions associated with the HPA can affect the diurnal rhythm of the cortisol and ACTH production and cause serious health problems.

The biologic effects of cortisol, including those caused by hypercortisolemia, can be modulated at the GR level using receptor modulators, such as agonists, partial agonists and antagonists. Several different classes of agents are able to block the physiologic effects of GR-agonist binding. These antagonists include compositions which, by binding to GR, block the ability of an agonist to effectively bind to and/or activate the GR. One such known GR antagonist, mifepristone, has been found to be an effective anti-glucocorticoid agent in humans (Bertagna (1984) J. Clin. Endocrinol. Metab. 59:25). Mifepristone binds to the GR with high affinity, with a dissociation constant (K_d) of 10^{-9} M (Cadepond (1997) Annu. Rev. Med. 48:129).

A variety of disease states are capable of being treated with glucocorticoid receptor modulators, including, e.g., mifepristone; glucocorticoid receptor modulators (e.g, glucocorticoid receptor antagonists) disclosed in U.S. Pat. No. 7,928,237 and in U.S. Pat. No. 8,461,172; glucocorticoid receptor modulators disclosed in U.S. Pat. No. 8,685,973; glucocorticoid receptor modulators disclosed in U.S. Patent Publication 2014/0038926 (now U.S. Pat. No. 8,859,774); and other glucocorticoid receptor modulators. Exemplary disease states include major psychotic depression, mild cognitive impairment, psychosis, dementia, hyperglycemia, stress disorders, antipsychotic induced weight gain, delirium, cognitive impairment in depressed patients, cognitive deterioration in individuals with Down's syndrome, psychosis associated with interferon-alpha therapy, chronic pain (e.g. pain associate with gastroesophageal reflux disease), postpartum psychosis, postpartum depression, neurological disorders in premature infants, migraine headaches, obesity, diabetes, cardiovascular disease, hypertension, Syndrome X, depression, anxiety, glaucoma, human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), neurodegeneration (e.g. Alzheimer's disease and Parkinson's disease), cognition enhancement, Cushing's Syndrome, Addison's Disease, osteoporosis, frailty, inflammatory diseases (e.g., osteoarthritis, rheumatoid arthritis, asthma and rhinitis), adrenal function-related ailments, viral infection, immunodeficiency, immunomodulation, autoimmune diseases, allergies, wound healing, compulsive behavior, multi-drug resistance, addiction, psychosis, anorexia, cachexia, post-traumatic stress syndrome post-surgical bone fracture, medical catabolism, and muscle frailty. The methods of treatment include administering to a patient in need of such treatment, a therapeutically effective amount of a glucocorticoid receptor modulator compound.

Cushing's syndrome is one of these problems. Patients having Cushing's syndrome usually have easy bruising; abdominal obesity and thin arms and legs; facial plethora; acne; proximal muscle weakness; and/or red purple stripes across the body. Cushing's syndrome is accompanied by hypercortisolemia, a condition involving a prolonged excess of circulating cortisol. Cushing's syndrome can be classified as exogenous Cushing's syndrome, which is caused by excess use of glucocorticoids drugs, such as prednisone, dexamethasone, and hydrocortisone, and endogenous Cushing's syndrome, which is caused by deregulatory abnormalities in the HPA axis. Endogenous Cushing's syndrome consists of the ACTH-independent Cushing's syndrome, characterized by an overproduction of cortisol in the absence of elevation of ACTH secretion; the ACTH-dependent Cushing's syndrome, characterized by excessive ACTH secretion.

ACTH-dependent Cushing's syndrome includes roughly 80% of patients having endogenous Cushing's syndrome and consists of two major forms: Cushing Disease and ectopic ACTH syndrome. The former is caused by a pituitary tumor and the latter is caused by a tumor outside the pituitary. Correct differential diagnosis between the Cushing Disease and ectopic ACTH syndrome is important for endocrinologists to recommend transphenoidal surgery or appropriate imaging to identify source of the ectopic ACTH secretion.

One current approach of differentially diagnosing patients with ACTH-dependent Cushing's syndrome involves measuring ACTH levels from samples obtained simultaneously from both inferior petrosal venous sinus (IPS)—a procedure referred to as inferior petrosal venous sinus sampling (IPSS)—and from the internal jugular or another peripheral vein. In one approach, referred herein as CRH-IPSS, 5 blood samples are taken from each IPS and the internal jugular vein, two before and three after administration of CRH. A central-to-periphery ACTH ratio of >2 before and >3 after the administration of CRH is consistent with Cushing Disease while a lower ratio favors ectopic ACTH syndrome. This procedure requires prolonged catheterization with the likelihood of infection, thrombosis, or bleeding rising with the duration of catheterization. In addition CRH is a protein which is expensive to produce, causing a shortage in supply between 2011 and early 2013, and requires sophisticated handling. Thus, the results from CRH-IPSS for differentially diagnosing patients with ACTH-dependent Cushing's syndrome often fall in the gray area. Desmopressin acetate (DDAVP), the alternative to CRH, which has also been used for IPSS, has similar disadvantages.

Another approach, referred to herein as metyrapone-IPSS, is similar to the one above, except that metyrapone instead of CRH is administered to the patient before IPSS and that samples are only taken from the patients after the metyrapone administration. Although metyrapone-IPSS improves the CRH-IPSS—since it dispenses with the need

for sampling before the administration of metyrapone, and thus reduces the duration of catheterization and likelihood of infection, thrombis, or bleeding associated therewith—it also has serious limitations. First, metyrapone acts to block the conversion of 11-deoxycortisol to cortisol by 11β-hydroxylase, causing a decrease in cortisol level, which in turn stimulates ACTH production and release. Since its effect on the ACTH secretion is indirect, the test result may be skewed by other factors affecting the cortisol synthesis. Second, as a cortisol synthesis blocker, treatment of metyrapone—especially at a high dose—may result in adrenal insufficiency or have deleterious effects on various normal bodily functions that require cortisol—for example, the anti-stress and anti-inflammation functions. Third, metyrapone is currently not available in the United States, consequently this diagnosis method is out of reach for many patients in this country.

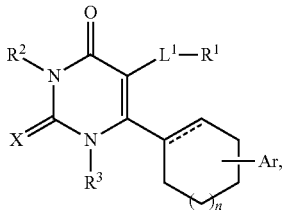
BRIEF SUMMARY OF THE INVENTION

In one aspect, provided herein is a method of differentially diagnosing adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome in a patient with hypercortisolemia where the differential diagnosis is between ectopic ACTH syndrome and Cushing Disease. The method comprises: (i) selecting a patient with Cushing's syndrome and elevated ACTH levels; (ii) administering a dose of glucocorticoid receptor antagonist (GRA) sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal HPA function; (iii) waiting for at least two hours; and (iv) obtaining from the patient an ACTH concentration ratio, which is derived both from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery vein, e.g., a jugular vein. The patient is diagnosed with Cushing Disease if the ACTH concentration ratio is greater than 3.

In some embodiments, the periphery venous sample is a jugular venous sample. In some embodiments, the ratio is derived from the ACTH concentration in fluid obtained from the left and right inferior petrosal venous sinuses. In some embodiments, the GRA is a selective inhibitor of the glucocorticoid receptor. In some cases, the first and second samplings of ACTH are taken 5-10 minutes apart from both the inferior petrosal venous sinus and a periphery venous sample.

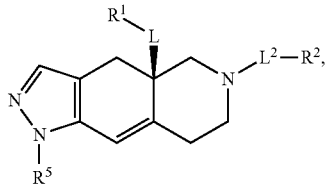
In some cases, the GRA is a selective inhibitor of the glucocorticoid receptor. In some embodiments, the GRA comprises a steroidal backbone with at least one phenyl-containing moiety in the 11-β position of the steroidal backbone. In some cases, the phenyl-containing moiety in the 11-β position of the steroidal backbone is a dimethylaminophenyl moiety. In some cases, the GRA is mifepristone. In some embodiments, the GRA is selected from the group consisting of 11β-(4-dimethylaminoethoxyphenyl)-17α-propynyl-17β-hydroxy-4,9 estradien-3-one and (17α)-17-hydroxy-19-(4-methylphenyl)androsta-4,9(11)-dien-3-one. In some embodiments, the glucocorticoid receptor antagonist is (11β,17β)-11-(1,3-benzodioxol-5-yl)-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one.

In some embodiments, the GRA has a non-steroidal backbone. In some cases, the GRA backbone is a cyclohexyl pyrimidine. In some cases, wherein the cyclohexyl pyrimidine has the following formula:

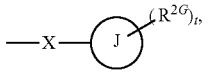


the dashed line is absent or a bond; X is selected from the group consisting of O and S; R^1 is selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl, and heteroaryl, optionally substituted with 1-3 R^{1a} groups; each R^{1a} is independently selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, C_{1-6} alkyl OR^{1b}, halogen, C_{1-6} haloalkyl, C_{1-6} haloaloxy, OR^{1b}, $NR^{1b}R^{1c}$, $C(O)R^{1b}$, $C(O)OR^{1b}$, $OC(O)R^{1b}$, $C(O)NR^{1b}R^{1c}$, $NR^{1b}C(O)R^{1c}$, S_2R^{1b}, $SO_2NR^{1b}R^{1c}$, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl; R^{1b} and R^{1c} are each independently selected from the group consisting of H and C_{1-6} alkyl; R^2 is selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkyl-OR^{1b}, C_{1-6} alkyl $NR^{1b}R^{1c}$ and C_{1-6} alkylene heterocycloalkyl; R^3 is selected from the group consisting of H and C_{1-6} alkyl; Ar is aryl, optionally substituted with 1-4 R^4 groups; each R^4 is independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, halogen, C_{1-6} haloalkyl, and C_{1-6} haloalkoxy; L^1 is a bond or C_{1-6} alkylene; and subscript n is an integer from 0 to 3, or salts and isomers thereof.

In some cases, the GRA backbone is a fused azadecalin. In some cases, the fused azadecalin is a compound having the following formula:



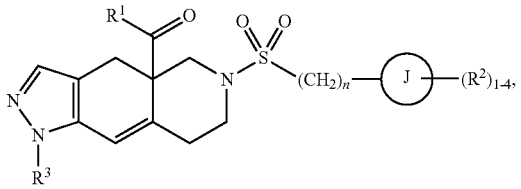
wherein L^1 and L^2 are members independently selected from a bond and unsubstituted alkylene; R^1 is a member selected from unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted heterocycloalkyl, —OR^{1A}, $NR^{1C}R^{1D}$, —C(O)$NR^{1C}R^{1D}$, and —C(O)OR^{1A}, wherein R^{1A} is a member selected from hydrogen, unsubstituted alkyl, and unsubstituted heteroalkyl; R^{1C} and R^{1D} are members independently selected from unsubstituted alkyl and unsubstituted heteroalkyl, and are optionally joined to form an unsubstituted ring with the nitrogen to which they are attached, wherein said ring optionally comprises an additional ring nitrogen. R^2 has the formula:



wherein R^{2G} is a member selected from hydrogen, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, —CN, and —CF_3; J is phenyl; t is an integer from 0 to 5; X is

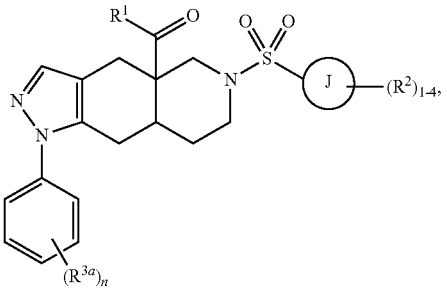
—$S(O_2)$—; and R^5 is phenyl optionally substituted with 1-5 R^{5A} groups, wherein R^{5A} is a member selected from hydrogen, halogen, —OR^{5A1}, $S(O_2)NR^{5A2}R^{5A3}$, —CN, and unsubstituted alkyl, and R^{5A1} is a member selected from hydrogen and unsubstituted alkyl, and R^{5A2} and R^{5A3} are members independently selected from hydrogen and unsubstituted alkyl, or salts and isomers thereof.

In some cases, the GRA backbone is a heteroaryl ketone fused azadecalin or an octahydro fused azadecalin. In some cases, the heteroaryl ketone fused azadecalin has the formula:



wherein R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S, optionally substituted with 1-4 groups each independently selected from R^{1a}; each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, CN, N-oxide, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl; ring J is selected from the group consisting of a cycloalkyl ring, a heterocycloalkyl ring, an aryl ring, and a heteroaryl ring, wherein the heterocycloalkyl and heteroaryl rings have from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O, and S; each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_1 6 haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, CN, OH, $NR^{2a}R^{2b}$, $C(O)R^{2a}$, $C(O)OR^{2a}$, $C(O)NR^{2a}R^{2b}$, SR^{2a}, $S(O)R^{2a}$, $S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl, wherein the heterocycloalkyl groups are optionally substituted with 1-4 R^{2c} groups; alternatively, two R^2 groups linked to the same carbon are combined to form an oxo group (=O); alternatively, two R^2 groups are combined to form a heterocycloalkyl ring having from 5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O, and S, wherein the heterocycloalkyl ring is optionally substituted with 1-3 R^{2d} groups; R^{2a} and R^{2b} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl; each R^{2c} is independently selected from the group consisting of hydrogen, halogen, hydroxy, C_{1-6} alkoxy, C_{1-6} haloalkoxy, CN, and $NR^{2a}R^{2b}$; each R^{2d} is independently selected from the group consisting of hydrogen and C_{1-6} alkyl, or two R^{2d} groups attached to the same ring atom are combined to form (=O); R^3 is selected from the group consisting of phenyl and pyridyl, each optionally substituted with 1-4 R^{3a} groups; each R^{3a} is independently selected from the group consisting of hydrogen, halogen, and C_{1-6} haloalkyl; and subscript n is an integer from 0 to 3; or salts and isomers thereof.

In some cases, the octahydro fused azadecalin has the formula:



wherein R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O, and S, optionally substituted with 1-4 groups each independently selected from R^{1a}; each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, N-oxide, and C_{3-8} cycloalkyl; ring J is selected from the group consisting of an aryl ring and a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O, and S; each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, CN, OH, $NR^{2a}R^{2b}$, $C(O)R^{2a}$, $C(O)OR^{2a}$, $C(O)NR^{2a}R^{2b}$, SR^{2a}, $S(O)R^{2a}$, $S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl having from 1 to 3 heteroatoms each independently selected from the group consisting of N, O, and S; alternatively, the two R^2 groups on adjacent ring atoms are combined to form a heterocycloalkyl ring having from 5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O, and S, wherein the heterocycloalkyl ring is optionally substituted with 1-3 R^{2c} groups; R^{2a}, R^{2b}, and R^{2c} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl; each R^{3a} is independently halogen; and subscript n is an integer from 0 to 3, or salts and isomers thereof.

In yet another aspect, provided herein is a diagnostic composition, or a diagnostic kit comprising a glucocorticoid receptor antagonist (GRA) for use in a method of differentially diagnosing adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome in a patient where the differential diagnosis is between ectopic ACTH syndrome and Cushing Disease, the method comprising the step of determining the ACTH concentration ratio from a patient with Cushing's syndrome and an elevated ACTH level, where the patient has been administered a dose of glucocorticoid receptor antagonist (GRA) at least two hours prior to the removal of venous samples and where the amount of GRA administered to the patient is sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function; wherein the ACTH concentration ratio is derived from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery venous sample; and wherein an ACTH concentration ratio of greater than 3 for the ACTH concentration from the inferior venous sinus sample over the periphery venous sinus sample is diagnostic indicative of Cushing's disease.

In yet another aspect, provided here in is a method of obtaining a measurement indicative of differential diagnosis of adrenocorticotropic hormone (ACTH)-dependent Cush-

ing's syndrome in a patient where the differential diagnosis is between ectopic ACTH syndrome and Cushing Disease, the method comprising the step of: (i) determining the ACTH concentration ratio from a patient with Cushing's syndrome and an elevated ACTH level, where the patient has been administered a dose of glucocorticoid receptor antagonist (GRA) at least two hours prior to the removal of venous samples and where the amount of GRA administered to the patient is sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function; wherein the ACTH concentration ratio is derived from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery venous sample; and wherein an ACTH concentration ratio of greater than 3 for the ACTH concentration from the inferior venous sinus sample over the periphery venous sinus sample is indicative of Cushing's disease.

In yet another aspect, provided herein is a glucocorticoid receptor antagonist (GRA) for use in a method of differentially diagnosing adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome in a patient where the differential diagnosis is between ectopic ACTH syndrome and Cushing Disease, the method comprising the steps of: (i) selecting a patient with Cushing's syndrome and also elevated ACTH levels; (ii) administering a dose of the GRA sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function; (iii) waiting for at least two hours; and (iv) obtaining from the patient an ACTH concentration ratio wherein the ratio is derived from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery venous sample; wherein an ACTH concentration ratio of greater than 3 for the ACTH concentration from the inferior venous sinus sample over the periphery venous sinus sample is diagnostic of Cushing's disease.

Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

This invention involves the use of GRAs to provide a robust and reproducible means to stimulate ACTH production in the pituitary gland for the differential diagnosis of patients with ACTH-dependent Cushing's syndrome, where the differential diagnosis is between ectopic ACTH syndrome and Cushing Disease. GRAs are first administered, and blood samples are then taken by IPSS after sufficient time for the assessment of ACTH levels.

The claimed methods have many advantages over the existing differential diagnosis methods, such as CRH-IPSS, DDAVP-IPSS and metyrapone-IPSS. First, the claimed methods are more robust compared to metyrapone-IPSS. GRAs used in the invention act to block cortisol binding to the receptor—thus preventing cortisol from inhibiting ACTH production and resulting in increased ACTH production/secretion. Compared to metyrapone, which acts to block the cortisol synthesis pathway, GRAs' effect on ACTH stimulation is more direct, thus making the test results more reliable. Second, compared to CRH/DDAVP-IPSS, the methods are cost-effective and convenient to use because GRAs are orally deliverable and less expensive than CRH to manufacture and store. Third, compared to CRH/DDAVP-IPSS, the method disclosed herein dispenses with the need to sample blood before the administration of GRAs, and thus reduces the duration of catheterization and minimizes complications associated with prolonged catheterization.

II. Definitions

The term "endogenous Cushing's syndrome" refers to a form of Cushing's syndrome, where the excess cortisol level is caused by the body's own overproduction of corti sol.

The term "Adrenocorticotropic hormone (ACTH)-dependent Cushings syndrome" refers to a form of endogenous Cushing's syndrome, which is caused by abnormal production of ACTH. There are two major forms of ACTH-dependent Cushing's syndrome: Cushing Disease (accounting for about 80% of the cases) and ectopic ACTH syndrome (accounting for 20% of the cases).

The term "ACTH concentration ratio", "ACTH ratio", "pituitary to periphery ACTH ratio", or "central to periphery ACTH ratio" disclosed herein refers to the ratio between the amount, level, or concentration of ACTH in the blood sample obtained from inferior petrosal sinus and the blood sample obtained from the periphery veins. In one embodiment, the periphery vein is the jugular vein.

The term "prolactin concentration ratio", "prolactin ratio", "pituitary to periphery prolactin ratio", or "central to periphery prolactin ratio" disclosed herein refers to the ratio between the amount, level, or concentration of prolactin in the blood sample obtained from inferior petrosal sinus and the blood sample obtained from the periphery veins. In one embodiment, the periphery vein is the jugular vein.

The term "differentially diagnosing" refers to the distinguishing of a particular disease or condition from others that present similar symptoms. A differential diagnostic method is a systematic diagnostic method used to identify the presence of a condition where multiple alternatives are possible. This method is essentially a process of elimination or a process of obtaining information that shrinks the "probabilities" of candidate conditions to negligible levels. The method uses evidence such as symptoms, test results, patient history, and medical knowledge to adjust epistemic confidences in the mind of the diagnostician (or, for computerized or computer-assisted diagnosis, the software of the system). Often each individual option of a possible disease is called a differential diagnosis.

The term "ectopic ACTH syndrome" refers to the abnormal production of ACTH due to ectopic ACTH secretion by an extrapituitary tumor. These extrapituitary tumors frequently originate in lungs, but in some cases originate from the thymus, pancreas, adrenal gland or thyroid.

The term "Cushing Disease" refers to the condition in which the pituitary gland releases too much ACTH as a result of a tumor located in—or excess growth (hyperplasia) of—the pituitary gland. Cushing Disease is a form of Cushing's syndrome.

The term "hypercortisolemia" refers a condition of having a higher than normal amount of circulating cortisol.

The term "inferior petrosal sinus sampling (IPSS)" refers to an invasive procedure performed to obtain blood samples from one or both petrosal venous sinuses by inserting catheters in one or both inferior petrosal veins via the jugular or femoral veins. The petrosal venous sinus drains the pituitary via the cavernous sinus. Thus, samples obtained from IPSS are often analyzed and compared with the

samples obtained from periphery blood for the amount of a particular analyte to detect signs of a disease relating to the pituitary gland.

The term "jugular venous sampling" refers to an invasive procedure performed to obtain blood samples from jugular veins (a periphery vein) by inserting catheters in the internal jugular vein via femoral veins. The tips of the catheters are typically advanced to the level of the angles of the mandible.

The term "periphery venous sinus sampling" refers to an invasive procedure performed to obtain blood samples from periphery veins by catheterization. Non-limiting examples of periphery veins include adrenal veins, high inferior vena cava, hepatic vein, azygos and hemiazygos veins, right atrium, right and left innominate and thymic veins, jugular veins, and both superior and middle thyroid veins.

The term "patient," "individual", or "subject" is used interchangeably to refer to a human subject. In some cases, the individual is suspected of having Cushing's Syndrome.

The term "administering" includes oral administration, topical contact, administration as a suppository, intravenous, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal, or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, epicutaneous, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, and transdermal patches.

The term "sample" refers to a biological sample obtained from a human subject. The sample can be any cell, tissue or fluid from a human subject. Samples can be subject to various treatment, storage or processing procedures before being analyzed according to the methods described herein. Generally, the terms "sample" or "samples" are not intended to be limited by their source, origin, manner of procurement, treatment, processing, storage or analysis, or any modification.

The term "cortisol" refers to a glucocorticoid hormone that is produced by the zona fasciculata of the adrenal gland.

The term "adrenocorticotropic hormone" or "ACTH" refers to a polypeptide-based hormone that is normally produced and secreted by the anterior pituitary gland. ACTH stimulates secretion of cortisol and other glucocorticoids (GCs) by specialized cells of the adrenal cortex. In healthy mammals, ACTH secretion is tightly regulated. ACTH secretion is positively regulated by corticotropin releasing hormone (CRH), which is released by the hypothalamus. ACTH secretion is negatively regulated by cortisol and other glucocorticoids.

The term "measuring the level," in the context of cortisol, ACTH, or other steroids, refers determining, detecting, or quantitating the amount, level, or concentration of, for example, cortisol, ACTH or other steroids in a sample obtained from a subject.

The term a "increase" or a "decrease" refers to a detectable positive or negative change in quantity from a comparison control, e.g., an established standard control (such as an average level of cortisol in a normal, healthy subject who does not have hypercortisolemia). An increase is a positive change that is typically at least 5%, at least 10%, or at least 20%, or 50%, or 100%, and can be as high as at least 1.5-fold, at least 2-fold, at least 5-fold, or even 10-fold of the control value. Similarly, a decrease is a negative change that is typically at least 5%, at least 10%, or at least 20%, 30%, or 50%, or even as high as at least 80% or 90% of the control value. Other terms indicating quantitative changes or differences from a comparative basis, such as "more," "less," "higher," and "lower," are used in this application in the same fashion as described above.

The term "normal reference value", "reference value", or "standard control level" refers to the a predetermined amount, level, or concentration of a particular analyte, e.g., ACTH, cortisol, or prolactin—by comparison to which a diagnosis of the presence or absence of a particular condition can be made, e.g., hypercortisolemia. Normal reference values referred to in this disclosure are in some cases provided by the commercial test that is used to determine the analyte levels. In some cases, a normal reference value, reference value, or standard control level is established as the average of the amount, level, or concentration of an analyte from one or more normal, healthy subjects, e.g., subjects who have normal HPA function. In some cases, they are established as a range of the level, amount, or concentration of the analyte in a group of healthy subjects. Normal reference values may vary depending on the nature of the sample, the manner or timing of sample collection, as well as other factors such as the sex, age, and ethnicity of the subjects for whom such a control value is established.

The term "elevated level", "elevated amount", or "elevated concentration" refers to the level or amount of the analyte that is higher than the normal reference value for that analyte.

The term "chromatography" refers to a process in which a chemical mixture carried by a liquid or gas is separated into components as a result of the differential distribution of the chemical entities as they flow around or over a stationary liquid or solid phase.

The term "liquid chromatography" or "LC" refers to a process of selective retardation of one or more components of a fluid solution when the fluid uniformly percolates either through a column of a finely divided substance or through capillary passageways. The retardation results from the distribution of the components of the mixture between one or more stationary phases and the bulk fluid, (i.e., mobile phase), as this fluid moves relative to the stationary phase(s). Examples of "liquid chromatography" include reverse phase liquid chromatography (RPLC), high performance liquid chromatography (HPLC), and turbulent flow liquid chromatography (TFLC) (sometimes known as high turbulence liquid chromatography (HTLC) or high throughput liquid chromatography).

The term "high performance liquid chromatography" or "HPLC" (also sometimes known as "high pressure liquid chromatography") refers to liquid chromatography in which the degree of separation is increased by forcing the mobile phase under pressure through a stationary phase—typically a densely packed column. As used herein, the term "ultra high performance liquid chromatography", "HPLC" or "UHPLC" (sometimes known as "ultra high pressure liquid chromatography") refers to HPLC which occurs at much higher pressures than in traditional HPLC techniques.

The term "glucocorticosteroid" ("GC") or "glucocorticoid" refers to a steroid hormone that binds to a glucocorticoid receptor. Glucocorticosteroids are typically characterized by having 21 carbon atoms, an α,β-unsaturated ketone in ring A, and an α-ketol group attached to ring D. They differ in the extent of oxygenation or hydroxylation at C-11, C-17, and C-19; see Rawn, "Biosynthesis and Transport of Membrane Lipids and Formation of Cholesterol Derivatives," in Biochemistry, Daisy et al. (eds.), 1989, pg. 567.

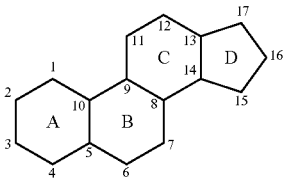
The term "glucocorticoid receptor" ("GR") refers to the type II GR which specifically binds to cortisol and/or cortisol analogs such as dexamethasone; See, e.g., Turner & Muller, *J Mol. Endocrinol,* 2005 (35): 283-292. The GR is also referred to as the cortisol receptor. The term includes isoforms of GR, recombinant GR and mutated GR. Inhibition constants (K_i) against the human GR receptor type II (Genbank: P04150) are between 0.0001 nM and 1,000 nM; preferably between 0.0005 nM and 10 nM, and most preferably between 0.001 nM and 1 nM.

The term "glucocorticoid receptor antagonist" or "GRA" refers to any composition or compound which partially or completely inhibits (antagonizes) the binding of a glucocorticoid receptor (GR) agonist, such as cortisol, or cortisol analogs, synthetic or natural, to a GR. A "specific glucocorticoid receptor antagonist" refers to any composition or compound which inhibits any biological response associated with the binding of a GR to an agonist. By "specific," the drug preferentially binds to the GR rather than to other nuclear receptors, such as the mineralocorticoid receptor (MR), androgen receptor (AR), or progesterone receptor (PR). It is preferred that the specific glucocorticoid receptor antagonist binds GR with an affinity that is 10× greater ($1/10^{th}$ the K_d value) than its affinity to the MR, AR, or PR, both the MR and PR, both the MR and AR, both the AR and PR, or to the MR, AR, and PR. In a more preferred embodiment, the specific glucocorticoid receptor antagonist binds a GR with an affinity that is 100× greater ($1/100^{th}$ the K_d value) than its affinity to the MR, AR, or PR, both the MR and PR, both the MR and AR, both the AR and PR, or to the MR, AR, and PR.

The term "selective inhibitor" in the context of a glucocorticoid receptor refers to a chemical compound that selectively interferes with the binding of a specific glucocorticoid receptor agonist and a glucocorticoid receptor.

The term "steroidal backbone" in the context of glucocorticoid receptor antagonists containing such refers to glucocorticoid receptor antagonists that contain modifications of the basic structure of cortisol, an endogenous steroidal glucocorticoid receptor ligand. The basic structure of a steroidal backbone is provided as Formula I:

Formula I



Steroidal Backbone

The two most commonly known classes of structural modifications of the cortisol steroid backbone to create glucocorticoid antagonists include modifications of the 11-β hydroxy group and modification of the 17-0 side chain (See, e. g., Lefebvre (1989) J. Steroid Biochem. 33: 557-563).

As used herein, the term "non-steroidal backbone" in the context of glucocorticoid receptor antagonists containing such refers to glucocorticoid receptor antagonists that do not share structural homology to, or are not modifications of, cortisol. Such compounds include synthetic mimetics and analogs of proteins, including partially peptidic, pseudopeptidic, and non-peptidic molecular entities.

Non-steroidal GRA compounds also include glucocorticoid receptor antagonists having a cyclohexyl-pyrimidine backbone, a fused azadecalin backbone, a heteroaryl ketone fused azadecalin backbone, or an octahydro fused azadecalin backbone. Exemplary glucocorticoid receptor antagonists having a cyclohexyl-pyrimidine backbone include those described in U.S. Pat. No. 8,685,973. Exemplary GRAs having a fused azadecalin backbone include those described in U.S. Pat. Nos. 7,928,237 and 8,461,172. Exemplary GRAs having a heteroaryl ketone fused azadecalin backbone include those described in U.S. Pat. Pub. 2014/0038926. Exemplary GRAs having an octohydro fused azadecalin backbone include those described in U.S. Provisional Patent Appl. No. 61/908,333, entitled Octahydro Fused Azadecalin Glucocorticoid Receptor Modulators, filed on Nov. 25, 2013.

Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g., —CH_2O— is equivalent to —OCH_2—.

"Alkyl" refers to a straight or branched, saturated, aliphatic radical having the number of carbon atoms indicated. Alkyl can include any number of carbons, such as C_{1-2}, C_{1-3}, C_{1-4}, C_{1-5}, C_{1-6}, C_{1-7}, C_{1-8}, C_{1-9}, C_{1-10}, C_{2-3}, C_{2-4}, C_{2-5}, C_{2-6}, C_{3-4}, C_{3-5}, C_{3-6}, C_{4-5}, C_{4-6}, and C_{5-6}. For example, C_{1-6} alkyl includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec.butyl, tert.butyl, pentyl, isopentyl, and hexyl.

"Alkoxy" refers to an alkyl group having an oxygen atom that connects the alkyl group to the point of attachment: alkyl-O—. As for the alkyl group, alkoxy groups can have any suitable number of carbon atoms, such as C_{1-6}. Alkoxy groups include, for example, methoxy, ethoxy, propoxy, iso-propoxy, butoxy, 2-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, pentoxy, hexoxy, etc.

"Halogen" refers to fluorine, chlorine, bromine, and iodine.

"Haloalkyl" refers to alkyl, as defined above, where some or all of the hydrogen atoms are replaced with halogen atoms. As for the alkyl group, haloalkyl groups can have any suitable number of carbon atoms, such as C_{1-6}, and include trifluoromethyl, fluoromethyl, etc.

The term "perfluoro" can be used to define a compound or radical where all the hydrogens are replaced with fluorine. For example, perfluoromethane includes 1,1,1-trifluoromethyl.

"Haloalkoxy" refers to an alkoxy group where some or all of the hydrogen atoms are substituted with halogen atoms. As for the alkyl group, haloalkoxy groups can have any suitable number of carbon atoms, such as C_{1-6}. The alkoxy groups can be substituted with 1, 2, 3, or more halogens. When all the hydrogens are replaced with a halogen, for example by fluorine, the compounds are per-substituted, for example, perfluorinated. Haloalkoxy includes, but is not limited to, trifluoromethoxy, 2,2,2,-trifluoroethoxy, and perfluoroethoxy.

"Cycloalkyl" refers to a saturated or partially unsaturated, monocyclic, fused bicyclic, or bridged polycyclic ring assembly containing from 3 to 12 ring atoms, or the number of atoms indicated. Cycloalkyl can include any number of carbons, such as C_{3-6}, C_{4-6}, C_{5-6}, C_{3-8}, C_{4-8}, C_{5-8}, C_{6-8}, C_{3-9}, C_{3-10}, C_{3-11}, and C_{3-12}. Saturated monocyclic cycloalkyl rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Saturated bicyclic and polycyclic cycloalkyl rings include, for example, norbornane, [2.2.2] bicyclooctane, decahydronaphthalene, and adamantane. Cycloalkyl groups can also be partially unsaturated, having one or more double or triple bonds in the ring.

Representative cycloalkyl groups that are partially unsaturated include, but are not limited to, cyclobutene, cyclopentene, cyclohexene, cyclohexadiene (1,3- and 1,4-isomers), cycloheptene, cycloheptadiene, cyclooctene, cyclooctadiene (1,3-, 1,4- and 1,5-isomers), norbornene, and norbornadiene. When cycloalkyl is a saturated monocyclic C_{3-8} cycloalkyl, exemplary groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. When cycloalkyl is a saturated monocyclic C_{3-6} cycloalkyl, exemplary groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

"Heterocycloalkyl" refers to a saturated ring system having from 3 to 12 ring members and from 1 to 4 heteroatoms of N, O, and S. Additional heteroatoms can also be useful, including but not limited to, B, Al, Si, and P. The heteroatoms can also be oxidized, such as, but not limited to, —S(O)— and —$S(O)_2$—. Heterocycloalkyl groups can include any number of ring atoms, such as 3 to 6, 4 to 6, 5 to 6, 3 to 8, 4 to 8, 5 to 8, 6 to 8, 3 to 9, 3 to 10, 3 to 11, or 3 to 12 ring members. Any suitable number of heteroatoms can be included in the heterocycloalkyl groups, such as 1, 2, 3, or 4, or 1 to 2, 1 to 3, 1 to 4, 2 to 3, 2 to 4, or 3 to 4. The heterocycloalkyl group can include groups such as aziridine, azetidine, pyrrolidine, piperidine, azepane, azocane, quinuclidine, pyrazolidine, imidazolidine, piperazine (1,2-, 1,3- and 1,4-isomers), oxirane, oxetane, tetrahydrofuran, oxane (tetrahydropyran), oxepane, thiirane, thietane, thiolane (tetrahydrothiophene), thiane (tetrahydrothiopyran), oxazolidine, isoxalidine, thiazolidine, isothiazolidine, dioxolane, dithiolane, morpholine, thiomorpholine, dioxane, or dithiane. The heterocycloalkyl groups can also be fused to aromatic or non-aromatic ring systems to form members including, but not limited to, indoline.

When heterocycloalkyl includes 3 to 8 ring members and 1 to 3 heteroatoms, representative members include, but are not limited to, pyrrolidine, piperidine, tetrahydrofuran, oxane, tetrahydrothiophene, thiane, pyrazolidine, imidazolidine, piperazine, oxazolidine, isoxazolidine, thiazolidine, isothiazolidine, morpholine, thiomorpholine, dioxane and dithiane. Heterocycloalkyl can also form a ring having 5 to 6 ring members and 1 to 2 heteroatoms, with representative members including, but not limited to, pyrrolidine, piperidine, tetrahydrofuran, tetrahydrothiophene, pyrazolidine, imidazolidine, piperazine, oxazolidine, isoxazolidine, thiazolidine, isothiazolidine, and morpholine.

"Aryl" refers to an aromatic ring system having any suitable number of ring atoms and any suitable number of rings. Aryl groups can include any suitable number of ring atoms, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 ring atoms, as well as from 6 to 10, 6 to 12, or 6 to 14 ring members. Aryl groups can be monocyclic, fused to form bicyclic or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. Other aryl groups include benzyl, that has a methylene linking group. Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl, or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl. Some other aryl groups have 6 ring members, such as phenyl. Aryl groups can be substituted or unsubstituted.

"Heteroaryl" refers to a monocyclic, fused bicyclic, or tricyclic aromatic ring assembly containing 5 to 16 ring atoms, where from 1 to 5 of the ring atoms are a heteroatom such as N, O, or S. Additional heteroatoms can also be useful, including but not limited to, B, Al, Si, and P. The heteroatoms can also be oxidized, such as, but not limited to, N-oxide, —S(O)—, and —$S(O)_2$—. Heteroaryl groups can include any number of ring atoms, such as 3 to 6, 4 to 6, 5 to 6, 3 to 8, 4 to 8, 5 to 8, 6 to 8, 3 to 9, 3 to 10, 3 to 11, or 3 to 12 ring members. Any suitable number of heteroatoms can be included in the heteroaryl groups, such as 1, 2, 3, 4, or 5; or 1 to 2, 1 to 3, 1 to 4, 1 to 5, 2 to 3, 2 to 4, 2 to 5, 3 to 4, or 3 to 5. Heteroaryl groups can have from 5 to 8 ring members and from 1 to 4 heteroatoms, or from 5 to 8 ring members and from 1 to 3 heteroatoms, or from 5 to 6 ring members and from 1 to 4 heteroatoms, or from 5 to 6 ring members and from 1 to 3 heteroatoms. The heteroaryl group can include groups such as pyrrole, pyridine, imidazole, pyrazole, triazole, tetrazole, pyrazine, pyrimidine, pyridazine, triazine (1,2,3-, 1,2,4-, and 1,3,5-isomers), thiophene, furan, thiazole, isothiazole, oxazole, and isoxazole. The heteroaryl groups can also be fused to aromatic ring systems, such as a phenyl ring, to form members including, but not limited to, benzopyrroles such as indole and isoindole, benzopyridines such as quinoline and isoquinoline, benzopyrazine (quinoxaline), benzopyrimidine (quinazoline), benzopyridazines such as phthalazine and cinnoline, benzothiophene, and benzofuran. Other heteroaryl groups include heteroaryl rings linked by a bond, such as bipyridine. Heteroaryl groups can be substituted or unsubstituted.

The heteroaryl groups can be linked via any position on the ring. For example, pyrrole includes 1-, 2-, and 3-pyrrole; pyridine includes 2-, 3- and 4-pyridine; imidazole includes 1-, 2-, 4- and 5-imidazole; pyrazole includes 1-, 3-, 4- and 5-pyrazole; triazole includes 1-, 4- and 5-triazole; tetrazole includes 1- and 5-tetrazole; pyrimidine includes 2-, 4-, 5- and 6-pyrimidine; pyridazine includes 3- and 4-pyridazine; 1,2,3-triazine includes 4- and 5-triazine; 1,2,4-triazine includes 3-, 5- and 6-triazine; 1,3,5-triazine includes 2-triazine; thiophene includes 2- and 3-thiophene; furan includes 2- and 3-furan; thiazole includes 2-, 4- and 5-thiazole; isothiazole includes 3-, 4- and 5-isothiazole; oxazole includes 2-, 4- and 5-oxazole; isoxazole includes 3-, 4- and 5-isoxazole; indole includes 1-, 2- and 3-indole; isoindole includes 1- and 2-isoindole; quinoline includes 2-, 3- and 4-quinoline; isoquinoline includes 1-, 3- and 4-isoquinoline; quinazoline includes 2- and 4-quinoazoline; cinnoline includes 3- and 4-cinnoline; benzothiophene includes 2- and 3-benzothiophene; and benzofuran includes 2- and 3-benzofuran.

Some heteroaryl groups include those having from 5 to 10 ring members and from 1 to 3 ring atoms including N, O, or S, such as pyrrole, pyridine, imidazole, pyrazole, triazole, pyrazine, pyrimidine, pyridazine, triazine (1,2,3-, 1,2,4- and 1,3,5-isomers), thiophene, furan, thiazole, isothiazole, oxazole, isoxazole, indole, isoindole, quinoline, isoquinoline, quinoxaline, quinazoline, phthalazine, cinnoline, benzothiophene, and benzofuran. Other heteroaryl groups include those having from 5 to 8 ring members and from 1 to 3 heteroatoms, such as pyrrole, pyridine, imidazole, pyrazole, triazole, pyrazine, pyrimidine, pyridazine, triazine (1,2,3-, 1,2,4- and 1,3,5-isomers), thiophene, furan, thiazole, isothiazole, oxazole, and isoxazole. Some other heteroaryl groups include those having from 9 to 12 ring members and from 1 to 3 heteroatoms, such as indole, isoindole, quinoline, isoquinoline, quinoxaline, quinazoline, phthalazine, cinnoline, benzothiophene, benzofuran and bipyridine. Still other heteroaryl groups include those having from 5 to 6 ring members and from 1 to 2 ring heteroatoms including N, O or S, such as pyrrole, pyridine, imidazole, pyrazole, pyrazine, pyrimidine, pyridazine, thiophene, furan, thiazole, isothiazole, oxazole, and isoxazole.

Some heteroaryl groups include from 5 to 10 ring members and only nitrogen heteroatoms, such as pyrrole, pyri-

dine, imidazole, pyrazole, triazole, pyrazine, pyrimidine, pyridazine, triazine (1,2,3-, 1,2,4- and 1,3,5-isomers), indole, isoindole, quinoline, isoquinoline, quinoxaline, quinazoline, phthalazine, and cinnoline. Other heteroaryl groups include from 5 to 10 ring members and only oxygen heteroatoms, such as furan and benzofuran. Some other heteroaryl groups include from 5 to 10 ring members and only sulfur heteroatoms, such as thiophene and benzothiophene. Still other heteroaryl groups include from 5 to 10 ring members and at least two heteroatoms, such as imidazole, pyrazole, triazole, pyrazine, pyrimidine, pyridazine, triazine (1,2,3-, 1,2,4- and 1,3,5-isomers), thiazole, isothiazole, oxazole, isoxazole, quinoxaline, quinazoline, phthalazine, and cinnoline.

"Heteroatoms" refers to O, S, or N.

"Salt" refers to acid or base salts of the compounds used in the methods of the present invention. Illustrative examples of pharmaceutically-acceptable salts are mineral acid (hydrochloric acid, hydrobromic acid, phosphoric acid, and the like) salts, organic acid (acetic acid, propionic acid, glutamic acid, citric acid, and the like) salts, and quaternary ammonium (methyl iodide, ethyl iodide, and the like) salts. It is understood that the pharmaceutically-acceptable salts are non-toxic. Additional information on suitable pharmaceutically-acceptable salts can be found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, which is incorporated herein by reference.

"Isomers" refers to compounds with the same chemical formula but which are structurally distinguishable.

"Tautomer" refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one form to another.

Descriptions of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to produce compounds which are not inherently unstable—and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions—such as aqueous, neutral, or physiological conditions.

"Pharmaceutically-acceptable excipient" and "pharmaceutically-acceptable carrier" refer to a substance that aids the administration of an active agent to—and absorption by—a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically-acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors and colors, and the like. One of ordinary skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

III. Detailed Descriptions of Embodiments

A. Method for Differential Diagnosis of ACTH-Dependent Cushing's Syndrome

1. Selecting Patients Having ACTH-Dependent Cushing's Syndrome

The methods disclosed herein is used to provide differential diagnosis between Cushing Disease and ectopic ACTH syndrome to patients who have already been diagnosed as having ACTH-dependent Cushing's syndrome. A diagnosis of ACTH-dependent Cushing's syndrome can be made based on observation of certain clinical symptoms, the detection of hypercortisolemia and elevated blood ACTH levels.

a. Clinical Symptoms

Eligible patients may exhibit one or more of the following symptoms: easy bruising; abdominal obesity and thin arms and legs; facial plethora; acne; proximal myopathy (or proximal muscle weakness); striae (especially if reddish purple and 1 cm wide); and thin skin. Patients may also frequently feel changes in mood; change in appetite, headaches; a chronic feeling of tiredness; osteoporosis; low potassium; diabetes, and high blood pressure; decreased concentration peripheral edema hypokalemia; decreased libido acne kidney stones; impaired memory (especially short term); and unusual infections. Females patients may have irregular menstruation, hirsutism, or female balding. Pediatric patients may have weight gain with decreasing growth velocity; abnormal genital virilization; short stature; and pseudoprecocious puberty or delayed puberty. The next step is to confirm these patients have hypercortisolemia.

b Hypercortisolemia

A diagnosis of hypercortisolemia requires the determination of the patient's circulating cortisol level. Various types of samples that can be used for this purpose, such as saliva, urine, whole blood, serum, and plasma. Samples may also be collected at different time during the day. In one approach, the patient's whole blood sample is collected and processed to collect serum, i.e., in the morning, e.g., at 8 am. or in the afternoon, e.g., at 4 pm. The collected serum sample is refrigerated or frozen within, e.g., 2 hours of collection. Analysis of the serum sample is performed in a timely fashion, e.g. within 7 days from sample collection. In another approach, the patient's cortisol levels are measured from his or her saliva samples. Salivary cortisol is in equilibrium with the free cortisol in blood circulation. Changes of cortisol levels in the bloodstream are paralleled, within minutes, by similar alterations in salivary cortisol concentrations, such that one can use the latter as a surrogate of the former. The commonly used saliva-based cortisol test is the midnight saliva test, which measures cortisol levels from saliva samples collected at between 11 pm and midnight. Intake of food or drink is prohibited at least 15 minutes prior to sample collection. Saliva samples are collected by keeping and rolling a swab in mouth for approximately 2 minutes. The saliva samples, ambient or refrigerated, are then sent to a laboratory for cortisol level determination in a timely fashion, e.g., within 7 days from sample collection.

Methods for measuring cortisol levels are known to those in the art. Useful assays include immunoassays, e.g., competitive immunoassay, radioimmunoassay, immunofluorometric enzyme assay, and ELISA, competitive protein-binding assay and mass spectrometry, e.g., high-performance liquid chromatography/triple quadrupole-mass spectrometry (LC-MS/MS). Commercial kits for measuring cortisol in samples are available from Beckman-Coulter, Seimens, Roche Diagnostics, and the like. Non-limiting examples of cortisol tests are Mayo Clinic's SALCT, CORT, CORTU, and CINP tests; an ADVIA Centaur® Cortisol assay (Siemens Healthcare Global); ARCHITECT i2000SR cortisol (Abbott); Immulite® 2000 Cortisol assay (Siemans Healthcare Global; #L2KCO2), Vitros ECi Cortisol assay (Ortho Clinical Diagnostics; #107 4053), and Elecsys® Cortisol Immunoassay (Roche Molecular Diagnostics; #11875116160).

The patient's cortisol measurement is then compared with the normal reference value; a level higher than the normal reference value indicates the patient has hypercortisolemia. The normal reference values for cortisol levels vary depending on the nature of the samples, the manner and timing of sample collection (higher for samples collected in the morning and lower for samples collected at night), and the detection method. Thus, it is essential to interpret test results in the context of the appropriate normal reference values. Various commercial kits provide the normal reference values in testing protocols. For example, normal reference values for the Mayo Clinic's SALCT test that determines cortisol level in saliva is <100 ng/dL; a saliva cortisol level higher than 100 ng/dL is thus an indication of hypercortisolemia. After being diagnosed with hypercortisolemia, the patient is subject to additional tests to confirm the presence of Cushing's syndrome.

c Cushing's Syndrome

At least one, preferably two or more, of the following tests are performed to diagnose Cushing's syndrome: 1) dexamethasone suppression test, which documents a loss of feedback inhibition of cortisol on the hypothalamic-pituitary-adrenal (HPA) axis; 2) 24-hour Urine Free Cortisol test, which assesses cortisol secretion in a 24-hour period; and 3) midnight salivary cortisol, which evaluates the loss of normal diurnal variation in cortisol secretion. If two of the three tests show abnormal cortisol levels, the Cushing's syndrome is confirmed.

The dexamethasone suppression test is typically used as a screen test for Cushing's syndrome. Dexamethasone is an exogenous steroid that binds glucocorticoid receptors in the anterior pituitary gland. When healthy individuals are treated with a low dose (1-2 mg) of dexamethasone, binding of dexamethasone to the glucocorticoid receptors provides negative feedback to the pituitary gland and results in suppression of ACTH secretion. The suppression of ACTH secretion, in turn, results in suppression of cortisol release and therefore a detectable decrease in cortisol level in circulation. In contrast, when patients having Cushing's syndrome are treated with a low dose of dexamethasone, no or little decrease in cortisol levels can be detected because of the excessive cortisol production associated with the disease. In one approach, the dexamethasone suppression test is performed by administering a low dose of dexamethasone, e.g., 1 mg, the night before at, e.g., 11 pm. The next morning, e.g., between 8-9 am; the patient's blood is then sampled and serum cortisol levels measured. Since normal subjects typically have serum cortisol levels reduced to less than 1.8 mg/dl, a serum cortisol level of more than 1.8 mg/dL is indicative of the presence of Cushing's syndrome,

The 24-hour Urine Free Cortisol test is the gold standard for diagnosing Cushing's syndrome. This test uses the principle that cortisol production is increased with Cushing's syndrome, and measurements of urinary excretion provide an integral estimate of that increase. A result more than the normal reference values is indicative of the presence of Cushing's syndrome. A 3 to 4-fold increase over normal reference values provides definite diagnosis of Cushing's syndrome; if this increase is present, no additional testing is required to confirm the diagnosis. For less dramatic increases in the urinary free-cortisol (UFC) level, other tests, such as the overnight dexamethasone suppression test and the midnight salivary cortisol test, as described above, are required.

The midnight saliva test is another test commonly used to confirm Cushing's syndrome. See the description of the test in the section above.

If the patient is confirmed to have Cushing's syndrome by two of the three tests, or by the detection of a 3 to 4-fold cortisol level increase in the 24-hour Urine Free Cortisol test, the next step is to measure ACTH to confirm he or she has ACTH-dependent Cushing's syndrome.

d ACTH-Dependent Cushing's Syndrome

There are two kinds of endogenous Cushing's syndrome: ACTH-dependent and ACTH-independent. The high cortisol level associated with ACTH-dependent Cushing's syndrome is caused by the overproduction of ACTH from a tumor, e.g., a pituitary tumor or an extrapituitary tumor. The excess cortisol level associated with ACTH-independent Cushing's syndrome, on the other hand, is caused by the overproduction of cortisol by a tumor in the adrenal gland or the overgrowth of the adrenal gland—either of which inhibits ACTH production and release. Thus, the ACTH levels are high in patients having ACTH-dependent Cushing's syndrome but low or even undetectable in patients having ACTH-independent Cushing's syndrome.

The biological samples that are suitable for ACTH determination can be serum, plasma, saliva, urine, or any other biological fluid taken from a subject. The sample can be the same or different from the sample used for cortisol level measurement. In some cases, the same sample that is used to measure cortisol level can be used to measure ACTH level. In other cases, different samples are used to measure cortisol and ACTH levels. For example, the cortisol levels can be measured in saliva and the ACTH levels can be measured in plasma. In yet other cases, different samples of the same type are used to measure the levels.

The level of ACTH can be measured using various methods, including but not limited to, immunoassays, e.g., competitive immunoassay, radioimmunoassay, immunofluorometric enzyme assay, and ELISA; competitive protein-binding assays; liquid chromatography (e.g., HPLC); and mass spectrometry, e.g., high-performance liquid chromatography/triple quadrupole-mass spectrometry (LC-MS/MS). Commercial kits for measuring ACTH are readily available, e.g., from Mayo clinic (Test ID: ACTH), Siemans Healthcare Global (Immulite® 2000 ACTH assay), and Roche Molecular Diagnostics (Catalog No. 03255751190).

A plasma ACTH concentration higher than the normal reference value indicates that the patient has ACTH-dependent Cushing's syndrome. Normal reference values vary depending on the assay method, type of sample, and timing of sample collection; like cortisol, ACTH in healthy individuals varies during a 24-hour period, reaching its highest level in the morning around 6-8 am and lowest at night around 11 pm. Various commercial kits provide the normal reference values in their testing protocols. For example, the normal reference values for Mayo Clinc Test ID: ACTH are about 10-60 pg/mL.

Patients diagnosed with ACTH-dependent Cushing's syndrome are selected, and the differential diagnosis performed as described below.

2. Method of Differential Diagnosis of ACTH-Dependent Cushing's Syndrome

The differential diagnosis method uses GRAs to discriminate between Cushing Disease and ectopic ACTH Cushing's syndrome, the two major forms of ACTH-dependent Cushing's syndrome. GRAs prevent cortisol from inhibiting both the CRH production in the hypothalamus and ACTH production in the pituitary gland through a negative feedback interaction, resulting in increased ACTH production and release. Patients with Cushing Disease have ACTH-producing tumors in the pituitary gland and thus will have a higher increase in ACTH level around the pituitary region than the

periphery region (outside the pituitary region). In contrast, patients with ectopic ACTH syndrome have the tumor growing outside the pituitary gland and thus will have a higher ACTH increase in the periphery than around the pituitary region. Thus a pituitary-to-periphery ratio can be used to discriminate between the two major types of ACTH-dependent Cushing's syndrome.

a Administration of GRA

GRA is administered at a dosage that is sufficient to increase ACTH in the pituitary gland by at least two fold in persons with normal HPA functions. In one embodiment, the GRA is mifepristone. In one embodiment, mifepristone is administered orally to the patient. In one embodiment, the mifepristone is administered at 300-1500 mg. In one embodiment, the GRA is administered at 11 pm the night before IPSS.

b. IPSS

The pituitary ACTH is measured from the blood sample obtained from the left, right, or both inferior petrosal sinuses (IPSs), which drain the pituitary gland. The periphery ACTH level is determined from the blood sample from a periphery vein. The procedure of sampling from inferior petrosal sinuses (known as IPSS) and the periphery is typically performed by an interventional radiologist.

IPSS is typically performed in the morning after administration of GRA, e.g., between 8 and 10 am, by advancing one or two microcatheters from the femoral vein up to one or both inferior petrosal sinuses. Meanwhile, another microcatheter is advanced to a periphery vein, e.g., the jugular vein. Venogram, or a digital venography, which documents the position of the catheters, is used to ensure the proper placement of the catheter; sampling begins only after confirming the microcatheter is positioned well in the IPS. Two samplings are made, at 5-10 minutes apart, by drawing blood simultaneously from the IPSs and the jugular vein at each sampling. Samples obtained are immediately placed in EDTA-containing tubes on ice. In some cases, an IPSS is performed only on one sinus, i.e., the left or right sinus. In some cases, the IPSS is performed for both sinuses (BIPSS). BIPSS provides values of ACTH from both right and left sinuses, a comparison of which provides useful information as to which side of the pituitary gland the tumor is located.

c. Diagnosis Based on the Central-to-Peripheral ACTH Ratio with Reference to Prolactin

The central-to-periphery ratio is the basis for the diagnosis; however the IPSS requires high level of expertise; since anomalous venous drainage, e.g., misplacement of the catherter tip when sampling the inferior petrosal sinus, may cause false negative results. In addition to IPSS venogram (described above), prolactin—which is also secreted by pituitary gland and circulated to the periphery—is often used as a marker for successful catheterization during IPSS. Prolactin levels are assessed from the same blood samples that are used for the ACTH analysis. A ratio of the central to periphery prolactin of more than 1.8 indicates successful catheterization.

Methods for measuring prolactin are known in the art. Useful assays include immunoassays, e.g., competitive immunoassay, radioimmunoassay, immunofluorometric enzyme assay, and ELISA; competitive protein-binding assay; and mass spectrometry, e.g., high-performance liquid chromatography/triple quadrupole-mass spectrometry (LC-MS/MS). Commerical kits for measuring prolactin are also readily available, e.g., from Abcam (Catalog # ab108655), R&D systems (Human Prolactin Quantikine ELISA Kit), and Cayman Chemical (Prolactin EIA Kit).

ACTH levels are determined using the methods described above. The patient's ACTH levels from one or both inferior petrosal sinuses are then compared with the ACTH levels in the periphery blood, and the petrosal sinus-to-periphery ACTH ratios are then determined. If the patient's inferior petrosal to periphery prolactin ratio is less than 1.8 (especially if less than 1.5)—an indication that the catheterization was improper—no diagnosis can be made and a new IPSS may need to be performed. If the patient's inferior petrosal-to-periphery prolactin ratio is more than 1.8 and the inferior petrosal-to-periphery ACTH ratio is greater than 3, he or she is then diagnosed as having Cushing Disease. If the patient's inferior petrosal-to-periphery prolactin ratio is more than 1.8 and the inferior petrosal-to periphery-ACTH ratio is less than 3, he or she is then diagnosed as having ectopic ACTH syndrome.

B. Establishing a Standard Control Level

As disclosed above, the differential diagnosis of ACTH dependent Cushing's syndrome involves comparisons of measurements of different hormones, e.g., prolactin, ACTH, and cortisol, with their respective normal reference values. In most cases, normal reference values, or standard control levels, are provided in the commercial kits that are used for the testing. Depending on circumstances, it may be necessary in some cases to establish a standard control level for the diagnosis. In order to establish a standard control for a particular sample type (e.g., a saliva sample, urine sample, plasma sample, or serum sample) for practicing the method of this disclosure, a group of healthy subjects, such as a group of subjects who do not have Cushing's Syndrome, is selected. These individuals are within the appropriate parameters, if applicable, for the purpose of diagnosing Cushing's Syndrome using the methods of the present invention. For instance, the individuals may be of similar age, gender, and comparable health status. Optionally, the individuals are of similar ethnic background.

The healthy status of the selected individuals can be confirmed by well-established, routinely employed methods, including but not limited to, general physical examination of the individuals and general review of their medical history.

Furthermore, the selected group of healthy individual must be of a reasonable size, such that the average amount, level, or concentration of cortisol, ACTH, or other steroid in the biological sample obtained from the group can be reasonably regarded as representative of the normal or average level among the general population of healthy individuals who do not experience Cushing's Syndrome. Preferably, the selected group comprises at least 10 normal, healthy human subjects.

Once an average value of cortisol, ACTH, or other steroid is established on the individual values found in each subject of the selected healthy control group, this average, median, or representative value or profile is considered a standard control level. A standard deviation is also determined during the same process. In some cases, separate standard control levels may be established for separately defined groups having distinct characteristics such as age, sex or ethnic background.

C. Glucocorticoid Receptor Antagonists

The methods of the present invention generally provide administering a GRA. In some cases, the glucocorticoid receptor antagonist is a specific GRA. As used herein, a specific glucocorticoid receptor antagonist refers to a composition or compound which inhibits any biological response associated with the binding of a glucocorticoid receptor to an agonist by preferentially binding to the glucocorticoid receptor rather than to another nuclear recep-

tor (NR). In some embodiments, the specific GRA binds preferentially to the glucocorticoid receptor rather than the mineralocorticoid receptor (MR), androgen receptor (AR), or progesterone receptor (PR). In an exemplary embodiment, the specific GRA binds preferentially to glucocorticoid receptor rather than the mineralocorticoid receptor (MR). In another exemplary embodiment, the specific GRA binds preferentially to the glucocorticoid receptor rather than the progesterone receptor (PR). In another exemplary embodiment, the specific GRA binds preferentially to the glucocorticoid receptor rather than the androgen receptor (AR). In yet another exemplary embodiment, the specific GRA binds preferentially to the glucocorticoid receptor in comparison to MR and PR, MR and AR, PR and AR, or MR, PR, and AR.

In a related embodiment, the specific GRA binds to the glucocorticoid receptor with an association constant (K_d) that is at least 10-fold less than the K_d for other nuclear receptors. In another embodiment, the specific GRA binds to the glucocorticoid receptor with an association constant (K_d) that is at least 100-fold less than the K_d for the other nuclear receptors. In another embodiment, the specific GRA binds to the glucocorticoid receptor with an association constant (K_d) that is at least 1000-fold less than the K_d for the other nuclear receptors.

Generally, treatment can be provided by administering an effective amount of a GRA of any chemical structure or mechanism of action and a glucocorticosteroid of any chemical structure or mechanism of action. Provided herein, are classes of exemplary GRAs and specific members of such classes. However, one of skill in the art will readily recognize other related or unrelated GRAs that can be employed in the treatment methods described herein.

1. GRAs Having a Steroidal Backbone

In some embodiments, an effective amount of a GRA with a steroidal backbone is administered to a subject for treatment of an ACTH-secreting tumor. Steroidal GRAs can be obtained by modification of the basic structure of glucocorticoid agonists, i.e., varied forms of the steroid backbone. The structure of cortisol can be modified in a variety of ways. The two most commonly known classes of structural modifications of the cortisol steroid backbone to create GRAs include modifications of the 11-β hydroxy group and modification of the 17-β side chain (See, e.g., Lefebvre, J. Steroid Biochem. 33:557-563, 1989).

Examples of steroidal GR antagonists include androgen-type steroidal compounds as described in U.S. Pat. No. 5,929,058, and the compounds disclosed in U.S. Pat. Nos. 4,296,206; 4,386,085; 4,447,424; 4,477,445; 4,519,946; 4,540,686; 4,547,493; 4,634,695; 4,634,696; 4,753,932; 4,774,236; 4,808,710; 4,814,327; 4,829,060; 4,861,763; 4,912,097; 4,921,638; 4,943,566; 4,954,490; 4,978,657; 5,006,518; 5,043,332; 5,064,822; 5,073,548; 5,089,488; 5,089,635; 5,093,507; 5,095,010; 5,095,129; 5,132,299; 5,166,146; 5,166,199; 5,173,405; 5,276,023; 5,380,839; 5,348,729; 5,426,102; 5,439,913; 5,616,458, 5,696,127, and 6,303,591. Such steroidal GR antagonists include cortexolone, dexamethasone-oxetanone, 19-nordeoxycorticosterone, 19-norprogesterone, cortisol-21-mesylate; dexamethasone-21-mesylate, 11β-(4-dimethylaminoethoxyphenyl)-17α-propynyl-17β-hydroxy-4,9-estradien-3-one (RU009), and (17α)-17-hydroxy-19-(4-methylphenyl)androsta-4,9(11)-dien-3-one (RU044).

Other examples of steroidal antiglucocorticoids are disclosed in Van Kampen et al. (2002) Eur. J. Pharmacol. 457(2-3):207, WO 03/043640, EP 0 683 172 B1, and EP 0 763 541 B1, each of which is incorporated herein by reference. EP 0 763 541 B1 and Hoyberg et al., Int'l J. of Neuro-psychopharmacology, 5:Supp. 1, S148 (2002) disclose the compound (11β,17β)-11-(1,3-benzodioxol-5-yl)-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one (ORG 34517), which in one embodiment, is administered in an amount effective to treat an ACTH-secreting tumor in a subject.

2. Removal or Substitution of the 11-β Hydroxy Group

Glucocorticoid antagonists with modified steroidal backbones comprising removal or substitution of the 11-β hydroxy group are administered in one embodiment of the invention. This class includes natural GRAs, including cortexolone, progesterone and testosterone derivatives, and synthetic compositions, such as mifepristone (Lefebvre, et al. supra). Preferred embodiments of the invention include all 11-β aryl steroid backbone derivatives because, in some cases, these compounds can be devoid of progesterone receptor (PR) binding activity (Agarwal, FEBS 217:221-226, 1987). In another embodiment an 11-β phenyl-aminodimethyl steroid backbone derivative, which is both an effective anti-glucocorticoid and anti-progesterone agent, is administered. These compositions can act as reversibly-binding steroid receptor antagonists. For example, when bound to a 11-β phenyl-aminodimethyl steroid, the steroid receptor can be maintained in a conformation that cannot bind its natural ligand, such as cortisol in the case of GR (Cadepond, 1997, supra).

Synthetic 11-beta phenyl-aminodimethyl steroids include mifepristone, also known as RU486, or 17-β-hydrox-11-β-(4-dimethyl-aminophenyl)17-α-(1-propynyl)estra-4,9-dien-3-one). Mifepristone has been shown to be a powerful antagonist of both the progesterone and glucocorticoid (GR) receptors. Thus, in some embodiments, the GRA administered to treat an ACTH-secreting tumor is mifepristone, or a salt, tautomer, or derivative thereof. In other embodiments, however, administration of mifepristone is specifically excluded as a GRA for treatment of an ACTH-secreting tumor.

Another 11-β phenyl-aminodimethyl steroid shown to have GR antagonist effects includes the dimethyl aminoethoxyphenyl derivative RU009 (RU39.009), 11-β-(4-dimethyl-aminoethoxyphenyl)-17-α-(propynyl-17-β-hydroxy-4,9-estradien-3-one) (see Bocquel, J. Steroid Biochem. Molec. Biol. 45:205-215, 1993). Another GR antagonist related to RU486 is RU044 (RU43.044) 17-β3-hydrox-17-α-19-(4-methyl-phenyl)-androsta-4,9(11)-dien-3-one) (Bocquel, 1993, supra). See also Teutsch, Steroids 38:651-665, 1981; U.S. Pat. Nos. 4,386,085 and 4,912,097.

One embodiment includes compositions that are irreversible anti-glucocorticoids. Such compounds include α-keto-methanesulfonate derivatives of cortisol, including cortisol-21-mesylate (4-pregnene-11-β, 17-α, 21-triol-3, 20-dione-21-methane-sulfonate and dexamethasone-21-mesylate (16-methyl-9-α-fluoro-1,4-pregnadiene-11β,17-α, 21-triol-3, 20-dione-21-methane-sulfonte). See Simons, J. Steroid Biochem. 24:25-32, 1986; Mercier, J. Steroid Biochem. 25:11-20, 1986; U.S. Pat. No. 4,296,206.

3. Modification of the 17-Beta Side Chain Group

Steroidal anti-glucocorticoids which can be obtained by various structural modifications of the 17-β side chain are also used in the methods of the invention. This class includes synthetic antiglucocorticoids, such as dexamethasone-oxetanone, various 17, 21-acetonide derivatives and 17-beta-carboxamide derivatives of dexamethasone (Lefebvre, 1989, supra; Rousseau, Nature 279:158-160, 1979).

4. Other Steroid Backbone Modifications

GRAs used in the various embodiments of the invention include any steroid backbone modification which effects a biological response resulting from a GR-agonist interaction. Steroid backbone antagonists can be any natural or synthetic variation of cortisol, such as adrenal steroids missing the C-19 methyl group, such as 19-nordeoxycorticosterone and 19-norprogesterone (Wynne, Endocrinology 107:1278-1280, 1980).

In general, the 11-β side chain substituent, and particularly the size of that substituent, can play a key role in determining the extent of a steroid's antiglucocorticoid activity. Substitutions in the A ring of the steroid backbone can also be important. For example, 17-hydroxypropenyl side chains can, in some cases, decrease antiglucocorticoid activity in comparison to 17-propynyl side chain containing compounds.

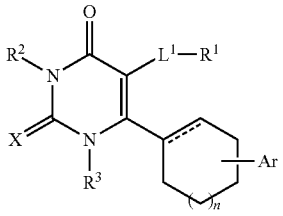
Additional glucocorticoid receptor antagonists known in the art and suitable for practice of the invention include 21-hydroxy-6,19-oxidoprogesterone (See Vicent, Mol. Pharm. 52:749-753, 1997), Org31710 (See Mizutani, J Steroid Biochem Mol Biol 42(7):695-704, 1992), RU43044, RU40555 (See Kim, J Steroid Biochem Mol Biol. 67(3): 213-22, 1998), and RU28362.

5. Non-Steroidal Anti-Glucocorticoids as Antagonists

Non-steroidal glucocorticoid receptor antagonists (GRAs) are also used in the methods of the invention to diagnose and treat Cushing's Syndrome in a subject. These include synthetic mimetics and analogs of proteins, including partially peptidic, pseudopeptidic and non-peptidic molecular entities. For example, oligomeric peptidomimetics useful in the invention include (α-β-unsaturated) peptidosulfonamides, N-substituted glycine derivatives, oligo carbamates, oligo urea peptidomimetics, hydrazinopeptides, oligosulfones and the like (See, e.g., Amour, Int. J. Pept. Protein Res. 43:297-304, 1994; de Bont, Bioorganic & Medicinal Chem. 4:667-672, 1996).

Examples of non-steroidal GR antagonists include the GR antagonist compounds disclosed in U.S. Pat. Nos. 5,696,127; 6,570,020; and 6,051,573; the GR antagonist compounds disclosed in US Patent Application 20020077356, the glucocorticoid receptor antagonists disclosed in Bradley et al., J. Med. Chem. 45, 2417-2424 (2002), e.g., 4α(S)-benzyl-2(R)-chloroethynyl-1,2,3,4,4α,9,10,10α(R)-octahydro-phenanthrene-2,7-diol ("CP 394531") and 4α(S)-benzyl-2(R)-prop-1-ynyl-1,2,3,4,4α,9,10,10α(R)-octahydro-phenanthrene-2,7-diol ("CP 409069"); and the compounds disclosed in PCT International Application No. WO 96/19458, which describes non-steroidal compounds that are high-affinity, highly selective antagonists for steroid receptors, such as 6-substituted-1,2-dihydro-N-protected-quinolines.

In some embodiments, Cushing's Syndrome is diagnosed and treated with an effective amount of a non-steroidal GRA having a cyclohexyl-pyrimidine backbone, a fused azadecalin backbone, a heteroaryl ketone fused azadecalin backbone, or an octahydro fused azadecalin backbone. For example, Cushing's Syndrome can be treated with effective amounts of one of the foregoing GRAs and a GC or a GC analog. Exemplary GRAs having a cyclohexyl-pyrimidine backbone include those described in U.S. Pat. No. 8,685,973. In some cases, the GRA having a cyclohexyl-pyrimidine backbone has the following structure:



wherein

the dashed line is absent or a bond;

X is selected from the group consisting of O and S;

R^1 is selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl and heteroaryl, optionally substituted with from 1 to 3 R^{1a} groups;

each R^{1a} is independently selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, C_{1-6} alkyl-OR^{1b}, halogen, C_{1-6} haloalkyl, C_{1-6} haloaloxy, —OR^{1b}, —$NR^{1b}R^{1c}$, —$C(O)R^{1b}$, —$C(O)OR^{1b}$, —$OC(O)R^{1b}$, —$C(O)NR^{1b}R^{1c}$, —$NR^{1b}C(O)R^{1c}$, —SO_2R^{1b}, —$SO_2NR^{1b}R^{1c}$, cycloalkyl, heterocycloalkyl, aryl and heteroaryl;

R^{1b} and R^{1c} are each independently selected from the group consisting of H and C_{1-6} alkyl;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkyl-OR^{1b}, C_{1-6} alkyl-$NR^{1b}R^{1c}$ and C_{1-6} alkylene-heterocycloalkyl;

R^3 is selected from the group consisting of H and C_{1-6} alkyl;

Ar is aryl, optionally substituted with 1-4 R^4 groups;

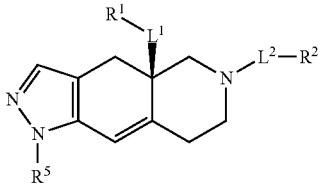
each R^4 is independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, halogen, C_{1-6} haloalkyl and C_{1-6} haloalkoxy;

L^1 is a bond or C_{1-6} alkylene; and

subscript n is an integer from 0 to 3,

or a salts and isomers thereof.

Exemplary GRAs having a fused azadecalin backbone include those described in U.S. Pat. Nos. 7,928,237; and 8,461,172. In some cases, the GRA having a fused azadecalin backbone has the following structure:



wherein

L^1 and L^2 are members independently selected from a bond and unsubstituted alkylene;

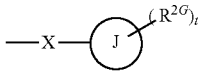
R^1 is a member selected from unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted heterocycloalkyl, —OR^{1A}, —$NR^{1C}R^{1D}$, —$C(O)NR^{1c}R^{1D}$, and —$C(O)OR^{1A}$, wherein

R^{1A} is a member selected from hydrogen, unsubstituted alkyl and unsubstituted heteroalkyl,

R^{1C} and R^{1D} are members independently selected from unsubstituted alkyl and unsubstituted heteroalkyl,

wherein R^{1C} and R^{1D} are optionally joined to form an unsubstituted ring with the nitrogen to which they are attached, wherein said ring optionally comprises an additional ring nitrogen;

R^2 has the formula:



wherein

R^{2G} is a member selected from hydrogen, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, —CN, and —CF_3;

J is phenyl;

t is an integer from 0 to 5;

X is —$S(O_2)$—; and

R^5 is phenyl optionally substituted with 1-5 R^{5A} groups, wherein

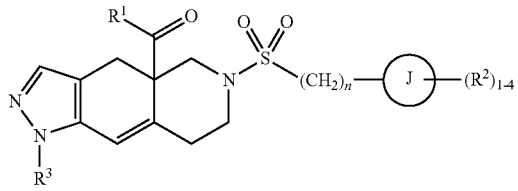
R^{5A} is a member selected from hydrogen, halogen, —OR^{5A1}, —$S(O_2)NR^{5A2}R^{5A3}$, —CN, and unsubstituted alkyl, wherein

R^{5A1} is a member selected from hydrogen and unsubstituted alkyl, and

R^{5A2} and R^{5A3} are members independently selected from hydrogen and unsubstituted alkyl,

or salts and isomers thereof.

Exemplary GRAs having a heteroaryl ketone fused azadecalin backbone include those described in U.S. 2014/0038926. In some cases, the GRA having a heteroaryl ketone fused azadecalin backbone has the following structure:



wherein

R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S, optionally substituted with 1-4 groups each independently selected from R^{1a};

each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, —CN, N-oxide, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl;

ring J is selected from the group consisting of a cycloalkyl ring, a heterocycloalkyl ring, an aryl ring and a heteroaryl ring, wherein the heterocycloalkyl and heteroaryl rings have from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S;

each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, —CN, —OH, —$NR^{2a}R^{2b}$, —$C(O)R^{2a}$, —$C(O)OR^{2a}$, —$C(O)NR^{2a}R^{2b}$, —SR^{2a}, —$S(O)R^{2a}$, —$S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl, wherein the heterocycloalkyl groups are optionally substituted with 1-4 R^{2c} groups;

alternatively, two R^2 groups linked to the same carbon are combined to form an oxo group (=O);

alternatively, two R^2 groups are combined to form a heterocycloalkyl ring having from 5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S, wherein the heterocycloalkyl ring is optionally substituted with from 1 to 3 R^{2d} groups;

R^{2a} and R^{2b} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

each R^{2c} is independently selected from the group consisting of hydrogen, halogen, hydroxy, C_{1-6} alkoxy, C_{1-6} haloalkoxy, —CN, and —$NR^{2a}R^{2b}$;

each R^{2d} is independently selected from the group consisting of hydrogen and C_{1-6} alkyl, or two R^{2d} groups attached to the same ring atom are combined to form (=O);

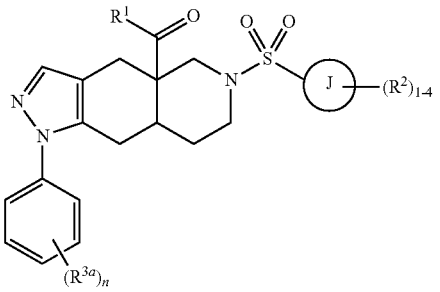
R^3 is selected from the group consisting of phenyl and pyridyl, each optionally substituted with 1-4 R^{3a} groups;

each R^{3a} is independently selected from the group consisting of hydrogen, halogen, and C_{1-6} haloalkyl; and

subscript n is an integer from 0 to 3;

or salts and isomers thereof.

Exemplary GRAs having an octohydro fused azadecalin backbone include those described in U.S. Provisional Patent Appl. No. 61/908,333, entitled Octahydro Fused Azadecalin Glucocorticoid Receptor Modulators, filed on Nov. 25, 2013. In some cases, the GRA having an octohydro fused azadecalin backbone has the following structure:



wherein

R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S, optionally substituted with 1-4 groups each independently selected from R^{1a};

each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, N-oxide, and C_{3-8} cycloalkyl;

ring J is selected from the group consisting of an aryl ring and a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S;

each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, —CN, —OH, —$NR^{2a}R^{2b}$, —$C(O)R^{2a}$, —$C(O)OR^{2a}$, —$C(O)NR^{2a}R^{2b}$, —SR^{2a}, —$S(O)R^{2a}$, —$S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl having from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S;

alternatively, two R^2 groups on adjacent ring atoms are combined to form a heterocycloalkyl ring having from

5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S, wherein the heterocycloalkyl ring is optionally substituted with from 1 to 3 R^{2c} groups;

R^{2a}, R^{2b} and R^{2c} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

each R^{3a} is independently halogen; and

subscript n is an integer from 0 to 3;

or salts and isomers thereof.

D. Pharmaceutical Compositions of Glucocorticoid Receptor Antagonists

The GRA compositions of the present disclosure can be prepared in a wide variety of oral, parenteral and topical dosage forms. Oral preparations of either include tablets, pills, powder, dragees, capsules, liquids, lozenges, cachets, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. The GRA compositions of the present invention can also be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally. Also, the GRA compositions described herein can be administered by inhalation, for example, intranasally. Additionally, the GRA compositions of the present invention can be administered transdermally. The GRA compositions of this invention can also be administered by intraocular, intravaginal, and intrarectal routes including suppositories, insufflation, powders and aerosol formulations (for examples of steroid inhalants, see Rohatagi, J. Clin. Pharmacol. 35:1187-1193, 1995; Tjwa, Ann. Allergy Asthma Immunol. 75:107-111, 1995). Accordingly, the present invention provides pharmaceutical compositions of a GRA including a pharmaceutically-acceptable carrier or excipient and a GRA compound of the present invention.

For preparing pharmaceutical compositions from the GRA compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances, which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, e.g., the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co, Easton Pa. ("Remington's").

In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% or 10% to 70% of the compounds of the present invention.

Suitable solid excipients include, but are not limited to, magnesium carbonate; magnesium stearate; talc; pectin; dextrin; starch; tragacanth; a low melting wax; cocoa butter; carbohydrates; sugars including, but not limited to, lactose, sucrose, mannitol, or sorbitol, starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; as well as proteins including, but not limited to, gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound (i.e., dosage). Pharmaceutical preparations of the invention can also be used orally using, for example, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain the compounds of the present invention mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the compounds of the present invention may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the compounds of the present invention are dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions suitable for oral use can be prepared by dissolving one or more compounds of the present invention in water and adding suitable colorants, flavors, stabilizers, and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be adjusted for osmolarity.

Also included are solid form preparations, which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Oil suspensions can be formulated by suspending the compounds of the present invention in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin; or a mixture of these. The oil suspensions can contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents can be added to provide a palatable oral preparation, such as

glycerol, sorbitol or sucrose. These formulations can be preserved by the addition of an antioxidant such as ascorbic acid. As an example of an injectable oil vehicle, see Minto, J. Pharmacol. Exp. Ther. 281:93-102, 1997. The pharmaceutical formulations of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil, described above, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion can also contain sweetening agents and flavoring agents, as in the formulation of syrups and elixirs. Such formulations can also contain a demulcent, a preservative, or a coloring agent.

The GRA compositions provided herein can also be delivered as microspheres for slow release in the body. For example, microspheres can be formulated for administration via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, J. Biomater Sci. Polym. Ed. 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao Pharm. Res. 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, J. Pharm. Pharmacol. 49:669-674, 1997). Both transdermal and intradermal routes afford constant delivery for weeks or months.

In another embodiment, the GRA compositions of the present invention can be formulated for parenteral administration, such as intravenous (IV) administration or administration into a body cavity or lumen of an organ. The formulations for administration will commonly comprise a solution of the compositions of the present invention dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These GRA formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of the compositions of the present invention in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV administration, the GRA formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol.

In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, i.e., by employing ligands attached to the liposome, or attached directly to the oligonucleotide, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, e.g., Al-Muhammed, J. *Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989).

Lipid-based drug delivery systems include lipid solutions, lipid emulsions, lipid dispersions, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS). In particular, SEDDS and SMEDDS are isotropic mixtures of lipids, surfactants and co-surfactants that can disperse spontaneously in aqueous media and form fine emulsions (SEDDS) or microemulsions (SMEDDS). Lipids useful in the formulations of the present invention include any natural or synthetic lipids including, but not limited to, sesame seed oil, olive oil, castor oil, peanut oil, fatty acid esters, glycerol esters, Labrafil®, Labrasol®, Cremophor®, Solutol®, Tween®, Capryol®, Capmul®, Captex®, and Peceol®.

The GRA composition can also contain other compatible therapeutic agents. The compounds described herein can be used in combination with one another, with other active agents known to be useful in antagonizing a glucocorticoid receptor, or with adjunctive agents that may not be effective alone, but may contribute to the efficacy of the active agent.

E. Administration

The GRA compounds or compositions of the present invention can be delivered by any suitable means, including oral, parenteral (e.g., intravenous injection or intramuscular injection) and topical methods. Transdermal administration methods, by a topical route, can be formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the compounds and compositions of the present invention. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

GRAs can be administered orally. For example, the GRA can be administered as a pill, a capsule, or liquid formulation as described herein. Alternatively, GRAs can be provided via parenteral administration. For example, the GRA can be administered intravenously (e.g., by injection or infusion). Additional methods of administration of the compounds described herein, and pharmaceutical compositions or formulations thereof, are described herein.

In some embodiments, the GRA is administered in one dose. In other embodiments, the GRA is administered in more than one dose, e.g., 2 doses, 3 doses, 4 doses, 5 doses, 6 doses, 7 doses, or more. In some cases, the doses are of an equivalent amount. In other cases, the doses are of different amounts. The doses can increase or taper over the duration of administration. The amount will vary according to, for example, the GRA properties. To determine an effective dose, the GRA must elevate the level of ACTH by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function.

In some embodiments, the subject diagnosed as having Cushing's Syndrome is administered a therapeutically effective amount of a GRA to ameliorate at least one symptom of Cushing's Syndrome. In some case, therapeutically effective amount of the GRA can be administered to treat Cushing's Syndrome.

IV. Examples

Example 1 Diagnosis of Hypercortisolemia

A 45-year-old female visits her endocrinologist. She appears to have abdominal obesity, thin arms and legs, a round red face, and a fat lump between the shoulders. She has acne and reddish purple stretch marks in the body that are more than 1 cm wide. She describes having fragile skin that heals poorly, irregular menstruation, and she often feels changes in mood, headaches, and a chronic feeling of tiredness. Her physical examination records show that she has proximal muscle weakness and osteoporosis. Her blood tests indicate that she has low potassium, diabetes and elevated blood pressure. She has not been taken exogenous glucocorticoid drugs prior to this visit. Her endocrinologist suspects she has hypercortisolemia, and orders a late night saliva cortisol test for her.

She complies to the requirement not to brush, eat, or drink for 30 minutes prior to the saliva collection. At midnight she collected her saliva by placing a swab into her mouth, while rolling the swab, for approximately 2 minutes. The sample is assayed using Mayo Clinic Test ID: SALCT following the protocol provided with the test. The result shows that her cortisol level is 200 ng/dL, indicating that she has hypercortisolemia.

Example 2. Diagnosis of Cushing's Syndrome

After diagnosis of hypercortisolemia, additional tests are ordered for her to determine whether she has Cushing's syndrome. First, a dexamethasone suppression test is performed. She is given 1 mg of dexamethasone at 11 pm, and the next morning her blood sample are collected between 8-9 am. Serum are collected from the blood and measured for cortisol using Mayo Clinic Test ID: CORT (http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8545), according to manufacturer's instructions. Her serum cortisol level is 2.2 mcg/dl, consistent with the presence of Cushing's syndrome.

Next, a 24 hour urine collection is ordered to measure her urine free cortisol. 3 mL of her 24-hour urine specimen is collected into a container, with the addition of 10 gram of boric acid as a preservative. The sample is centrifuged and removed of precipitate before the assay. Cortisol content is analyzed using Mayo Clinic Test ID: COCOU, according to manufacturer's instructions (http://www.mayomedicallaboratories.com/test-catalog/Specimen/82948). The test shows a cortisol level of 180 mcg—4 fold of the upper limit of the normal range of cortisol for the test: 3.5-45 mcg. Based on her 24-hour urine excretion test result as well as her clinical symptoms, she is diagnosed as having Cushing's syndrome. The next step is to measure ACTH to differentiate between ACTH-dependent and ACTH-independent Cushing's syndrome.

Example 3. Diagnosis of ACTH-Dependent Cushing's Syndrome

A blood test is then performed to determine her plasma ACTH level. 1 mL of whole blood sample is drawn from her in the morning. The blood is spun down in a refrigerated centrifuge and the plasma is immediately separated from cells. 0.5 mL of the plasma sample is assayed for ACTH using Mayo Clinic Test ID: ACTH, following the manufacturer's instructions (http://www.mayomedicallaboratories.com/test-catalog/Specimen/8411). The result shows her plasma ACTH is 80 pg/mL, which indicates that she has ACTH-dependent Cushing's syndrome.

Example 4. Diagnosis of Cushing Disease

Following the diagnosis of ACTH dependent Cushing's syndrome, she then undergoes IPSS to identify the source of abnormal ACTH secretion, i.e., whether it is pituitary or ectopic. Mifepristone administration and IPSS are performed to determine the cause of her ACTH-dependent Cushing's syndrome. She first takes an oral dose of 300-1500 mg of mifepristone at 11 pm the night before IPSS. Mifepristone at this dose is sufficient to increase ACTH from the pituitary gland by at least two-fold in persons having normal hypothalamic-pituitary-adrenal axis (HPA) function. Between 8 to 10 am, an interventional radiologist performs a femoral microcatheterization, in which two 0.018 inch microcatheters are advanced from the femoral vein up to her right and left inferior petrosal sinuses (IPS). Another 0.018 microcatherter is inserted into the peripheral jugular vein. A 5,000 unit bolus of heparin is administered to the veins to prevent venous sinus thrombosis.

After the microcathers enter the sinuses and the jugular bulb, a diagnostic venography is performed, in which a rapid injection of contrast is performed to attempt to reflux contrast into the inferior petrosal sinus to guide placement of a microcatheter. After confirming the position of the microcatheter and positioning it well in the IPS, two samplings are made at 5-10 minutes apart. Blood samples are drawn simultaneously from the IPS and the jugular vein at each sampling and immediately placed in EDTA-containing tubes on ice.

One half of each blood sample is centrifuged for 10 minutes at 1,000-2,000 g to remove the cells and collect plasma. The other half is left undisturbed at room temperature for 30 minutes to clot, and serum is obtained after removal of the clot by a centrifugation. The plasma samples from both the jugular vein and the IPS are assayed for ACTH using Mayo Clinic's Test ID: ACTH, as described above. The serum samples are assayed for prolactin using Mayo Clinic's Test ID: PLPMA, following the manufacturer's instructions. The results show that the prolactin level in her left IPS is 25 ng/ml and right IPS is 24 ng/ml. The prolactin level in her jugular vein is 12 ng/ml. The ACTH level in her IPS is 800 pg/ml and the ACTH in her jugular vein is 200 pg/ml.

Her IPSs (both left and right) to jugular vein prolactin ratio is greater than 1.8, which reflects the correct central-to-periphery gradient, thus confirming the correct positioning of the catheterization. Her IPSs to jugular vein ACTH ratio is greater than 3, which indicates she has Cushing Disease.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference.

What is claimed is:

1. A method of concurrently treating Cushing's syndrome and differentially diagnosing adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome in a patient where the differential diagnosis is between ectopic ACTH syndrome and Cushing's disease, the method comprising the steps of:

(i) selecting a patient with Cushing's syndrome and also elevated ACTH levels;

(ii) administering a dose of glucocorticoid receptor antagonist (GRA) sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function;

(iii) waiting for at least two hours; and,

(iv) obtaining from the patient an ACTH concentration ratio wherein the ratio is derived from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery venous sample;

wherein an ACTH concentration ratio of greater than 3 for the ACTH concentration from the inferior venous sinus sample over the periphery venous sinus sample is diagnostic of Cushing's disease.

2. The method of claim **1** wherein the periphery venous sample is a jugular venous sample.

3. The method of claim **1** wherein the glucocorticoid receptor antagonist is a selective inhibitor of the glucocorticoid receptor.

4. The method of claim **1** wherein a first and second sampling of the ACTH concentrations in the are taken 5-10 minutes apart from both the inferior petrosal venous sinus and a periphery venous sample.

5. The method of claim **1**, wherein the glucocorticoid receptor antagonist comprises a steroidal backbone with at least one phenyl-containing moiety in the 11-β position of the steroidal backbone.

6. The method of claim **5** wherein the phenyl-containing moiety in the 11-β position of the steroidal backbone is a dimethylaminophenyl moiety.

7. The method of claim **5**, wherein the glucoocoricoid receptor antagonist is mifepristone.

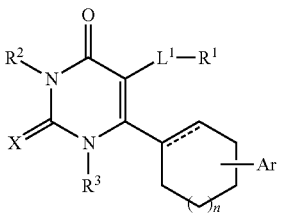
8. The method of claim **1**, wherein the glucocorticoid receptor antagonist is selected from the group consisting of 11β-(4-dimethylaminoethoxyphenyl)-17α-propynyl-17β-hydroxy-4,9 estradien-3-one and (17α)-17-hydroxy-19-(4-methylphenyl)androsta-4,9(11)-dien-3-one.

9. The method of claim **1**, wherein the glucocorticoid receptor antagonist is (11β,17β)-11-(1,3-benzodioxol-5-yl)-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one.

10. The method of claim **1**, wherein the glucocorticoid receptor antagonist has a non-steroidal backbone.

11. The method of claim **10**, wherein the glucocorticoid receptor antagonist backbone is a cyclohexyl pyrimidine.

12. The method of claim **11**, wherein the cyclohexyl pyrimidine has the following formula:



wherein

the dashed line is absent or a bond;

X is selected from the group consisting of O and S;

R^1 is selected from the group consisting of cycloalkyl, heterocycloalkyl, aryl and heteroaryl, optionally substituted with from 1 to 3 R^{1a} groups;

each R^{1a} is independently selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} alkoxy, C_{1-6} alkyl OR^{1b}, halogen, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, OR^{1b}, $NR^{1b}R^{1c}$, $C(O)R^{1b}$, $C(O)OR^{1b}$, $OC(O)R^{1b}$, $C(O)NR^{1b}R^{1c}$, $NR^{1b}C(O)R^{1c}$, SO_2R^{1b}, $SO_2NR^{1b}R^{1c}$, cycloalkyl, heterocycloalkyl, aryl and heteroaryl;

R^{1b} and R^{1c} are each independently selected from the group consisting of H and C_{1-6} alkyl;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkyl-OR^{1b}, C_{1-6} alkyl $NR^{1b}R^{1c}$ and C_{1-6} alkylene heterocycloalkyl;

R^3 is selected from the group consisting of H and C_{1-6} alkyl;

Ar is aryl, optionally substituted with 1-4 R^4 groups;

each R^4 is independently selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} alkoxy, halogen, C_{1-6} haloalkyl and C_{1-6} haloalkoxy;

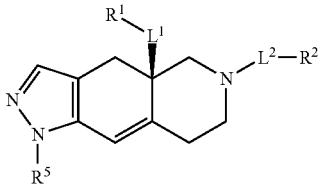
L^1 is a bond or C_{1-6} alkylene; and

subscript n is an integer from 0 to 3,

or salts thereof.

13. The method of claim **10**, wherein the glucocorticoid receptor antagonist backbone is a fused azadecalin.

14. The method of claim **13**, wherein the fused azadecalin is a compound having the following formula:



wherein

L^1 and L^2 are members independently selected from a bond and unsubstituted alkylene;

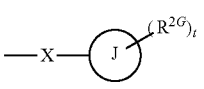
R^1 is a member selected from unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted heterocycloalkyl, —OR^{1A}, $NR^{1C}R^{1D}$, —$C(O)NR^{1C}R^{1D}$, and —$C(O)OR^{1A}$, wherein

R^{1A} is a member selected from hydrogen, unsubstituted alkyl and unsubstituted heteroalkyl,

R^{1C} and R^{1D} are members independently selected from unsubstituted alkyl and unsubstituted heteroalkyl,

wherein R^{1C} and R^{1D} are optionally joined to form an unsubstituted ring with the nitrogen to which they are attached, wherein said ring optionally comprises an additional ring nitrogen;

R^2 has the formula:



wherein

R^{2G} is a member selected from hydrogen, halogen, unsubstituted alkyl, unsubstituted heteroalkyl,

unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, —CN, and —CF_3;

J is phenyl;

t is an integer from 0 to 5;

X is —$S(O_2)$—; and

R^5 is phenyl optionally substituted with 1-5 R^{5A} groups, wherein

R^{5A} is a member selected from hydrogen, halogen, —OR^{5A1}, $S(O_2)NR^{5A2}R^{5A3}$, —CN, and unsubstituted alkyl, wherein

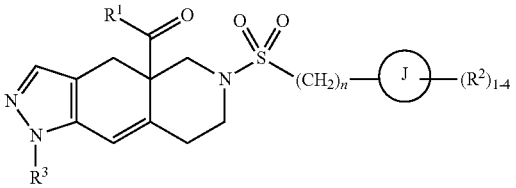
R^{5A1} is a member selected from hydrogen and unsubstituted alkyl, and

R^{5A2} and R^{5A3} are members independently selected from hydrogen and unsubstituted alkyl,

or salts thereof.

15. The method of claim **10**, wherein the glucocorticoid receptor antagonist backbone is a heteroaryl ketone fused azadecalin or an octahydro fused azadecalin.

16. The method of claim **15**, wherein the heteroaryl ketone fused azadecalin has the formula:



wherein

R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S, optionally substituted with 1-4 groups each independently selected from R^{1a};

each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, CN, N-oxide, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl;

ring J is selected from the group consisting of a cycloalkyl ring, a heterocycloalkyl ring, an aryl ring and a heteroaryl ring, wherein the heterocycloalkyl and heteroaryl rings have from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S;

each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, CN, OH, $NR^{2a}R^{2b}$, $C(O)R^{2a}$, $C(O)OR^{2a}$, $C(O)NR^{2a}R^{2b}$, SR^{2a}, $S(O)R^{2a}$, $S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl, wherein the heterocycloalkyl groups are optionally substituted with 1-4 R^{2c} groups;

alternatively, two R^2 groups linked to the same carbon are combined to form an oxo group (=O);

alternatively, two R^2 groups are combined to form a heterocycloalkyl ring having from 5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S, wherein the heterocycloalkyl ring is optionally substituted with from 1 to 3 R^{2d} groups;

R^{2a} and R^{2b} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

each R^{2c} is independently selected from the group consisting of hydrogen, halogen, hydroxy, C_{1-6} alkoxy, C_{1-6} haloalkoxy, CN, and $NR^{2a}R^{2b}$;

each R^{2d} is independently selected from the group consisting of hydrogen and C_{1-6} alkyl, or two R^{2d} groups attached to the same ring atom are combined to form (=O);

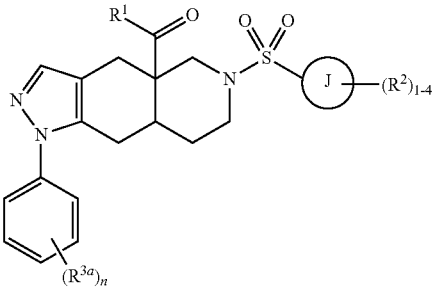
R^3 is selected from the group consisting of phenyl and pyridyl, each optionally substituted with 1-4 R^{3a} groups;

each R^{3a} is independently selected from the group consisting of hydrogen, halogen, and C_{1-6} haloalkyl; and

subscript n is an integer from 0 to 3;

or salts thereof.

17. The method of claim **15**, wherein the octahydro fused azadecalin has the formula:



wherein

R^1 is a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S, optionally substituted with 1-4 groups each independently selected from R^{1a};

each R^{1a} is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, N-oxide, and C_{3-8} cycloalkyl;

ring J is selected from the group consisting of an aryl ring and a heteroaryl ring having from 5 to 6 ring members and from 1 to 4 heteroatoms each independently selected from the group consisting of N, O and S;

each R^2 is independently selected from the group consisting of hydrogen, C_{1-6} alkyl, halogen, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkyl-C_{1-6} alkoxy, CN, OH, $NR^{2a}R^{2b}$, $C(O)R^{2a}$, $C(O)OR^{2a}$, $C(O)NR^{2a}R^{2b}$, SR^{2a}, $S(O)R^{2a}$, $S(O)_2R^{2a}$, C_{3-8} cycloalkyl, and C_{3-8} heterocycloalkyl having from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S;

alternatively, two R^2 groups on adjacent ring atoms are combined to form a heterocycloalkyl ring having from 5 to 6 ring members and from 1 to 3 heteroatoms each independently selected from the group consisting of N, O and S, wherein the heterocycloalkyl ring is optionally substituted with from 1 to 3 R^{2c} groups;

R^{2a}, R^{2b} and R^{2c} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

each R^{3a} is independently halogen; and

subscript n is an integer from 0 to 3,

or salts thereof.

18. A method of concurrently treating Cushing's syndrome and obtaining a measurement indicative of differen-

tial diagnosis of adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome in a patient where the differential diagnosis is between ectopic ACTH syndrome and Cushing's disease, the method comprising the steps of:

determining the ACTH concentration ratio from a patient with Cushing's syndrome and an elevated ACTH level,

where the patient has been administered a dose of glucocorticoid receptor antagonist (GRA) at least two hours prior to the removal of venous samples and

where the amount of GRA administered to the patient is sufficient to increase ACTH from the pituitary gland by at least two fold in persons with normal Hypothalamus Pituitary Adrenal (HPA) function;

wherein the ACTH concentration ratio is derived from the ACTH concentrations in fluid obtained from either the left or right inferior petrosal venous sinus and from fluid obtained from a periphery venous sample; and

wherein an ACTH concentration ratio of greater than 3 for the ACTH concentration from the inferior venous sinus sample over the periphery venous sinus sample is indicative of Cushing's disease.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,829,495 B2
APPLICATION NO. : 15/236015
DATED : November 28, 2017
INVENTOR(S) : Andreas G. Moraitis

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In Column 33, Claim 4, Line 30, remove "in the";

In Column 33, Claim 7, Line 40, remove "glucoocoricoid" and insert --glucocorticoid--;

In Column 35, Claim 16, Line 50, remove "halogen, $C_{1\ 6}$" and insert --halogen, C_{1-6}--; and,

In Column 35, Claim 16, Line 51, remove "$C_{1\ 6}$ alkoxy" and insert --C_{1-6} alkoxy--.

Signed and Sealed this
Twenty-sixth Day of December, 2017

Joseph Matal

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office

EXHIBIT C

US009943526B2

(12) **United States Patent**
Belanoff et al.

(10) **Patent No.: US 9,943,526 B2**
(45) **Date of Patent: Apr. 17, 2018**

(54) **OPTIMIZING MIFEPRISTONE LEVELS FOR CUSHING'S PATIENTS**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/133,791**

(22) Filed: **Apr. 20, 2016**

(65) **Prior Publication Data**

US 2016/0310507 A1 Oct. 27, 2016

Related U.S. Application Data

(60) Provisional application No. 62/150,757, filed on Apr. 21, 2015.

(51) **Int. Cl.**

A61K 31/122	(2006.01)
A61K 31/135	(2006.01)
A61K 31/136	(2006.01)
A61K 31/567	(2006.01)
G01N 33/94	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/567*** (2013.01); ***A61K 31/122*** (2013.01); ***A61K 31/135*** (2013.01); ***A61K 31/136*** (2013.01); ***G01N 33/94*** (2013.01); *G01N 2800/048* (2013.01); *G01N 2800/52* (2013.01)

(58) **Field of Classification Search**

CPC .. A61K 31/567; A61K 31/122; A61K 31/135; A61K 31/136

See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

The present invention provides a method for optimizing levels of mifepristone in a patient suffering from Cushing's syndrome. The method comprises the steps of treating the patient with seven or more daily doses of mifepristone over a period of seven or more days; testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1631 ng/mL; and adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1631 ng/mL.

9 Claims, No Drawings

(56) **References Cited**

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OPTIMIZING MIFEPRISTONE LEVELS FOR CUSHING'S PATIENTS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/150,757, filed Apr. 21, 2015, which is incorporated in its entirety herein for all purposes.

BACKGROUND OF THE INVENTION

It has been reported previously that administration of the same dose of mifepristone can produce widely varying blood serum levels in different patients. The varied blood serum levels can result in some patients not receiving an efficacious dose of mifepristone. For patients suffering from a mental disorder, the blood serum levels need to be maintained at about 1300 ng/mL. For patients suffering from Cushing's syndrome, it was surprisingly discovered that blood serum levels need to be maintained at a level of at least about 1631 ng/mL for a therapeutic response. Thus, a method for ensuring that the blood serum levels of mifepristone remain in an efficacious and safe range is needed for patients suffering from Cushing's syndrome.

BRIEF SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a method for improving efficacy of mifepristone treatment in a patient suffering from Cushing's syndrome. The method includes treating the patient with seven or more daily doses of mifepristone over a period of seven or more days; testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1631 ng/mL; and adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1631 ng/mL. The patient of the present invention is not already suffering from a condition indicated for treatment with mifepristone. Thus, the method thereby improves the efficacy of mifepristone treatment for patients suffering from Cushing's syndrome for the patient suffering from Cushing's syndrome.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

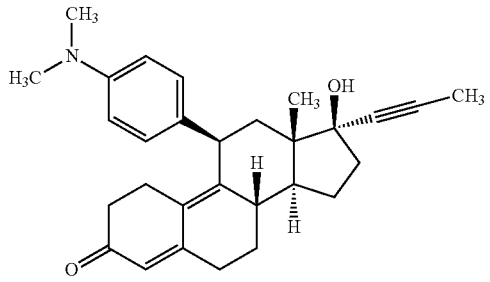
Administration of the same dose of mifepristone can produce widely varying mifepristone blood serum levels in different patients. For the same dose, the blood serum levels can differ by as much as 800% from one patient to another. For those patients with lower blood serum levels, the effectiveness of mifepristone treatment can suffer significantly. The present invention provides a method for optimizing the blood serum levels of mifepristone so that the blood serum levels remain in an efficacious range and the patient receives the necessary treatment.

The method of the present invention optimizes blood serum levels of mifepristone in a patient suffering from Cushing's syndrome by first treating the patient with mifepristone. The treatment can be for any appropriate period of time, such as seven or more daily doses over a period of seven or more days. Following treatment for an appropriate period of time, the serum levels of the patient are tested to determine whether the blood levels of mifepristone are greater than 1631 ng/mL. The daily dose of the patient is then adjusted in order to achieve mifepristone blood levels of greater than 1631 ng/mL.

Previous methods of optimizing mifepristone levels are known for patients suffering from mental disorders. But the earlier methods describe a minimum mifepristone blood level of only 1300 ng/mL. While patients with Cushing's syndrome are known to have higher cortisol levels, it is surprising that higher mifepristone blood level of 1631 ng/mL would be necessary to achieve optimal efficacy in treating Cushing's syndrome.

II. Definitions

"Mifepristone" refers to a compound having the following structure:



The term mifepristone also refers to a family of compositions also known as: RU486 or RU38.486; 17-beta-hydroxy-11-beta-(4-dimethyl-aminophenyl)-17-alpha-(1-propynyl)-estra-4,9-dien-3-one); 11-beta-(4dimethylaminophenyl)-17-beta-hydroxy-17-alpha-(1-propynyl)-estra-4,9-dien-3-one); 11B[p-(Dimethylamino) phenyl]-17B-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one; 11B-(4-dimethyl-aminophenyl)-17B-hydroxy-17A-(prop-1-ynyl)-estra-4,9-dien-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A-(propynyl-1)-estra-4,9-diene-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A-(propynyl-1)-E; (11B,17B)-11-[4-dimethylamino)-phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one; and 11B-[4-(N,N-dimethylamino) phenyl]-17A-(prop-1-ynyl)-D-4,9-estradiene-17B-ol-3-one. Salts, hydrates and prodrug forms of mifepristone are also useful in the formulations of the present invention.

Mifepristone and its analogs bind to the glucocorticoid receptor (GR), typically with high affinity, and inhibit the biological effects initiated/mediated by the binding of any cortisol or cortisol analogue to the GR. As such, mifepristone has been used to treat conditions associated with elevated cortisol levels including, for example, hyperadrenocorticism, also known as Cushing's syndrome (Chrousos, pp 273-284, In: Baulieu, ed. *The Antiprogestin Steroid RU 486 and Human Fertility Control*. Plenum Press, New York (1989), Sartor (1996) *Clin. Obstetrics and Gynecol.* 39:506-510). Patients with some forms of psychiatric illnesses can be responsive to treatments which block the effect of cortisol, as by administering GR antagonists (Van Look (1995) *Human Reproduction Update* 1:19-34). In one study, a patient with depression associated with Cushing's Syndrome was responsive to a high dose, up to 1400 mg per day, of mifepristone (Nieman (1985) *J. Clin Endocrinol. Metab.* 61:536). Due to its antiprogestogenic activity, mifepristone has also been employed in emergency contraception, medical abortion, and treatment of uterine fibroids and meningioma (Healy (2009) *Australian Prescriber* 32:152-154).

"Patient" refers to animals such as mammals, including, but not limited to, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice and the like. The patient can have a condition known to be treated by glucocorticoid antagonists such as mifepristone. Such conditions include, but are not limited to, psychiatric illnesses and hormonal disorders. In certain embodiments, the patient is a human. The patient can be male or female.

"Cushing's syndrome" refers to an endocrine disease with an estimated incidence of approximately 10-15 per 1 million persons (Meier and Biller (1997) *Endocrinol Metab Clin North Am* 26:741-762), and is associated with an increased blood concentration of cortisol (hypercortisolism) over a long period of time. Cushing's syndrome is classified as either ACTH dependent or non ACTH dependent. ACTH dependent Cushing's syndrome is characterized by a chronic ACTH hypersecretion which stimulates the growth of the adrenal glands and the hypersecretion of corticosteroids. The most common underlying cause of ACTH dependent Cushing's syndrome is excessive production of ACTH by pituitary adenomas known as Cushing's disease. Cushing's syndrome resulting from the production of ACTH in another location than the pituitary gland is known as ectopic Cushing's syndrome. Examples of ectopic sites include thymoma, medullary carcinoma of the thyroid, pheochromocytoma, islet cell tumors of the pancreas and small cell carcinoma of the lung. ACTH independent Cushing's syndromes are caused by adrenal tumors that can be either adenomas or carcinomas. Both adrenal adenomas and carcinomas are characterized by chronic cortisol hypersecretion.

"Optimizing" refers to the process of testing mifepristone blood levels and adjusting the dosage of mifepristone administered to the patient in need in order to achieve mifepristone blood levels above 1631 ng/mL.

"Treat", "treating" and "treatment" collectively refer to any indicia of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being; or, in some situations, preventing the onset of dementia. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation.

"Testing" refers to determining the mifepristone blood levels in a patient. The testing can be performed by any suitable instrument, such as a plasma sampling collection device capable of detecting mifepristone serum levels.

A patient "not already suffering from a condition indicated for treatment with mifepristone" is a patient who is not suffering from a condition which is known in the art to be effectively treatable with mifepristone. Conditions known in the art to be effectively treatable with mifepristone include drug withdrawal, psychosis, dementia, stress disorders, and psychotic major depression.

III. Method Of Optimizing Mifepristone Levels

The present invention provides a method of optimizing mifepristone levels in patients with Cushing's syndrome such that the blood serum levels remain at efficacious levels. The method involves administering mifepristone for a week, testing the blood serum levels of the Cushing's patient, and adjusting the mifepristone dose to maintain the mifepristone blood serum levels of at least 1631 ng/mL.

The present invention provides a method for improving efficacy of mifepristone treatment in a patient suffering from Cushing's syndrome. The method includes treating the patient with seven or more daily doses of mifepristone over a period of seven or more days; testing the serum levels of the patient to determine whether the blood levels of mifepristone are greater than 1631 ng/mL; and adjusting the daily dose of the patient to achieve mifepristone blood levels greater than 1631 ng/mL. The patient treated in this method is not already suffering from a condition indicated for treatment with mifepristone, thereby improving the efficacy of mifepristone treatment for the patient suffering from Cushing's syndrome.

The seven or more daily doses of mifepristone can each be administered by any means suitable, as described in more detail below. In some embodiments, each of the seven or more daily doses of mifepristone are administered orally.

The seven or more daily doses of mifepristone can each be administered in any suitable dose. For example, the mifepristone can be administered in an amount of at least about 100 mg. The mifepristone can also be administered in an amount of about 300, 600, 900 or about 1200 mg. In some embodiments, the daily dose can be at least 300 mg. In some embodiments, the daily dose can be at least 600 mg. In some embodiments, the daily dose can be at least 900 mg. In some embodiments, the daily dose can be at least 1200 mg. Other daily doses are useful in the method of the present invention.

The daily doses can be administered for any suitable period of time that is at least 7 days in length. For example, the daily doses can be for 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days. The mifepristone can be administered for longer periods as required by the patient being treated. In some embodiments, the patient can be treated with 28 or more daily doses over a period of 28 or more days.

The mifepristone blood levels can be tested by any means known to one of skill in the art. For example, the the testing can be performed by a plasma sampling collection device suitable for detecting mifepristone serum levels.

The mifepristone blood levels can be at any suitable level to treat Cushing's syndrome. For example, the mifepristone blood levels can be greater than about 1400 ng/mL, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800 or great than about 2900 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1450 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1469 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1600 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1631 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1662 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1666 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1700 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1800 ng/mL. In some embodiments, the mifepristone blood level can be greater than 1820 ng/mL. In some embodiments, the mifepristone blood level can be greater than 2000 ng/mL. In some embodiments, the mifepristone blood level can be greater than 2022 ng/mL.

The daily dose can be adjusted to any suitable dose to maintain the mifepristone blood level above the necessary level. For example, if the mifepristone blood level is below 1631 ng/mL, the daily dose can be increased to 600 mg from 300 mg, to 900 mg from 600 mg, to 900 mg from 300 mg, to 1200 mg from 900 mg, to 1200 mg from 600 mg, or to

1200 mg from 300 mg. If after another seven daily doses, the mifepristone blood level is still not above the necessary level, the mifepristone daily can again be increased. For example, the mifepristone daily dose can be increased to 900 mg from 600 mg, to 1200 mg from 900 mg, or to 1200 mg from 600 mg. In some embodiments, the adjusting step comprises increasing the daily dose of the patient to achieve mifepristone blood levels greater than 1631 ng/mL. Additional adjustments in the daily doses can be made to maintain the mifepristone blood level above 1631 ng/mL.

Any suitable percentage of the patient population can have the optimal response to administration of the mifepristone. For example, at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% of the patient population can achieve the optimal response to the mifepristone treatment. In some embodiments, at least about 20% of the patient population can have the optimal response to administration of the mifepristone. In some embodiments, at least about 40% of the patient population can have the optimal response to administration of the mifepristone. In some embodiments, at least about 60% of the patient population can have the optimal response to administration of the mifepristone.

A. Patients in Need

Patients amenable to treatment with mifepristone according to the method of the present invention suffer from Cushing's syndrome. Cushing's syndrome is a disorder resulting from increased adrenocortical secretion of corticosteroid. Hyperfunction of the adrenal cortex may be adrenocorticotropic hormone (ACTH)-dependent or it may be independent of ACTH regulation, e.g. production of corticosteroid by an adrenocortical adenoma or carcinoma. A common cause of Cushing's syndrome is excessive production of ACTH by the pituitary gland. This elevated level of ACTH in the bloodstream typically is produced by a pituitary adenoma (Cushing's disease), but in rare instances has a different etiology. Cushing's syndrome resulting from the production of ACTH in a location other than the pituitary gland is known as ectopic Cushing's syndrome. Examples of ectopic sites include thymoma, medullary carcinoma of the thyroid, pheochromocytoma, islet cell tumors of the pancreas and oat cell carcinoma of the lung. The overwhelming majority of Cushing's syndrome cases in humans, however, trace their etiology to a pituitary adenoma. Symptoms of Cushing's syndrome include weight gain, central obesity, steroid hypersecretion, elevated urinary cortisol excretion, moon face, weakness, fatigue, backache, headache, impotence, mental status changes, muscle atrophy, and increased thirst and urination compared to mammals not suffering from this disease. Diagnosis and treatment of Cushing's syndrome remains a challenge (see Oldfield, E. W. et al., N. Engl. J. Med., 325:897-905 (1991); Findling, J. W. et al., "Diagnosis and differential diagnosis of Cushing's syndrome," Endocrinol. Metab. Clin. North Am., 30:729-47 (2001); Orth, D. N., "Cushing's syndrome," N Engl J. Med., 332:791-803 (1995)). In experienced specialized centers, surgical resection of ACTH-secreting pituitary microadenomas offers an overall cure rate of about 70-80%, but for macroadenomas cure rates only approximate 30%, and the extensive surgical resection required portends significant risk to surrounding normal pituitary tissue, leading to partial or total hypopituitarism in about 80% of cases (Simmons, N. E. et al., "Serum Cortisol response to transphenoidal surgery for Cushing disease," J. Neurosurg., 95:1-8 (2001); Mampalam, T. J. et al., "Transsphenoidal microsurgery for Cushing's disease: A report of 216 cases," Ann. Intern. Med., 109:487-93 (1988); and Trainer, P. J. et al., "Transsphenoidal resection in Cushing's disease: undetectable serum cortisol as the definition of successful treatment," Clin. Endocrinol., 38:73-8 (1993)).

B. Formulations of Mifepristone

Formulations of the present invention include mifepristone in combination with pharmaceutical excipients. Mifepristone is commercially available from a variety of sources such as Eurolabs Ltd. (London, England). Mifepristone can also be synthesized by one of skill in the art using known synthetic procedures.

Mifepristone refers to a family of compositions also referred to as RU486, or RU38.486, or 17-beta-hydroxy-11-beta-(4-dimethyl-aminophenyl)-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or 11-beta-(4dimethylaminophenyl)-17-beta-hydroxy-17-alpha-(1-propynyl)-estra-4,9-dien-3-one), or analogs thereof, which bind to the GR, typically with high affinity, and inhibit the biological effects initiated/mediated by the binding of any cortisol or cortisol analogue to a GR receptor. Chemical names for RU-486 vary; for example, RU486 has also been termed: 11B[p-(Dimethylamino)phenyl]-17B-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one; 11B-(4-dimethyl-aminophenyl)-17B-hydroxy-17A-(prop-1-ynyl)-estra-4,9-dien-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A-(propynyl-1)-estra-4,9-diene-3-one; 17B-hydroxy-11B-(4-dimethylaminophenyl-1)-17A-(propynyl-1)-E; (11B,17B)-11-[4-dimethylamino)-phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one; and 11B-[4-(N,N-dimethylamino) phenyl]-17A-(prop-1-ynyl)-D-4,9-estradiene-17B-ol-3-one. Salts, hydrates and prodrug forms of mifepristone are also useful in the formulations of the present invention.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of mifepristone suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g., sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents. Preferred pharmaceutical preparations can deliver the compounds of the invention in a sustained release formulation.

C. Administration of Mifepristone

The formulations of the present invention provide serum levels of mifepristone of at least 1631 ng/mL. The mifepristone utilized in the pharmaceutical method of the invention

is administered at the initial dosage of about 0.001 mg/kg to about 1000 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the patient and the condition being treated. The dose administered to a patient, in the context of the present invention, should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound in a particular patient. Determination of the proper dosage for a particular situation is within the skill of the practitioner.

Generally, treatment is initiated with six daily doses, with the blood levels tested on the day of the seventh daily dose in order to determine whether the dose used is providing a mifepristone blood level of at least 1631 ng/mL. The testing is also performed to ensure the blood levels are below those afforded by an LD50 dose of about 1000 mg/kg. If the mifepristone blood level is lower than 1631 ng/mL. Additional testing of mifepristone blood levels can be necessary in order to confirm a mifepristone blood level of at least 1631 ng/mL or to adjust the mifepristone daily dose higher. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired. In addition, the interval from initiation of treatment and testing for mifepristone blood levels can be as short as 1 daily dose, or up to 28 daily doses and longer.

Mifepristone can be administered for any period of time, such as 7 daily doses over a period of seven days. Mifepristone can also be administered using more daily doses over a longer period of time, such as via 28 daily doses over a period of 28 days. Longer times for administration of mifepristone are also within the scope of the present invention.

D. Assay for Testing Mifepristone Levels

Mifepristone levels can be determined by any method known in the art. Methods for detecting mifepristone levels include, but are not limited to, radio-immuno assay and mass spectrometry (MALDI, SELDI, LS/MS, LS/MS/MS, among others). Liquid chromatography mass spectrometry (LC/MS or LC-MS) separates compounds chromatographically before they are introduced to the ion source and mass spectrometer. It differs from GC/MS in that the mobile phase is liquid, usually a combination of water and organic solvents, instead of gas. Most commonly, an electrospray ionization source is used in LC/MS.

Tandem mass spectrometry (MS/MS) involves multiple steps of mass selection or analysis, usually separated by some form of fragmentation. A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry. For example, one mass analyzer can isolate one peptide from many entering a mass spectrometer. A second mass analyzer then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A third mass analyzer then catalogs the fragments produced from the peptides. Tandem MS can also be done in a single mass analyzer over time as in a quadrupole ion trap. There are various methods for fragmenting molecules for tandem MS, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD) and blackbody infrared radiative dissociation (BIRD). One of skill in the art will appreciate that other assays for testing mifepristone levels are known to one of skill in the art.

In some embodiments, the assay can be performed as follows. Blood is collected from a patient in a vacutainer containing sodium heparin. The blood is centrifuged and the resulting plasma frozen at an appropriate temperature until assay. In some embodiments, the temperature is about −70° C. In other embodiments, other blood components can be collected and stored. Prior to analysis, the plasma is thawed and a fraction of the plasma is mixed with an internal standard in a solvent such as acetonitrile, to obtain a fixed concentration of the standard. In some embodiments, the internal standard can be mifepristone-d_4. The concentration of the internal standard is selected in order to be greater than the expected concentration of mifepristone in the plasma. For example, the internal standard can have a concentration of 2000 ng/mL. One of skill in the art will appreciate that other internal standards, and other concentrations, are useful in the present invention.

Base is then added to the sample solution. The base can be any amine or ammonium base, such as ammonium hydroxide. One of skill in the art will appreciate that other bases are useful in the present invention.

Solvent is then added to the solution and the mifepristone (along with the internal standard) are extracted from the plasma. Solvents useful for the extraction of mifepristone include, but are not limited to, hexanes, pentanes, ethers (such as diethylether, tetrahydrofuran and methyl-t-butyl ether (MTBE)), ethyl acetate, chloroform and methylene chloride. One of skill in the art will appreciate that other solvents are useful in the present invention.

Following separation and concentration of the organic layer, the sample is reconstituted in a solvent mixture comprising water, acetonitrile and formic acid. The ratio of the solvent components can vary. In some embodiments, the solvent mixture is water:acetonitrile:formic acid (75:25:0.1, v/v/v). One of skill in the art will appreciate that other solvent mixtures are useful in the present invention.

The sample can then be analyzed by reverse-phase high pressure liquid chromatography (HPLC). In some embodiments, the reverse-phase HPLC is performed using a water: acetonitrile:formic acid (60:40:0.1) mobile phase (isocratic) at a flow rate of 0.3 mL/min. One of skill in the art will appreciate that other mobile phases and flow rates are useful in the present invention.

The reverse-phase HPLC column can be a phenyl column maintained at 50° C. Mifepristone elutes at 4.2 minutes. Following elution, the mobile phase can be nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds detected using a tandem quadrupole mass spectrometer. Mifepristone (molecular weight of 430 g/mol) can be detected at m/z 372.30. The internal standard mifepristone-d_4 can be detected at m/z 376.30. The ratio of the mifepristone peak height to the peak height for the internal standard can then be calculated.

The plasma concentration of mifepristone is then calculated by comparing the experimental ratio to a standard curve of mifepristone:mifepristone-d_4 peak height ratio v. mifepristone concentration. The standard curve is generated by first measuring the mifepristone:mifepristone-d_4 peak height ratios for mifepristone samples at 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL where the mifepristone-d_4 internal standard has a concentration of 2000 ng/mL. The mifepristone:mifepristone-d_4 peak height ratios of these known solutions are then fit to a power equation (Mass Lynx

by Micromass, Beverly, Mass.), against which future samples with unknown concentrations of mifepristone are compared.

The plasma levels of mifepristone derivatives such as RU42633, RU42698 and RU42848, among others, can also be determined using the assay described above.

E. Kits for Treating Cushing's Syndrome with Mifepristone

The present invention provides kits. The kits of the present invention comprise seven daily doses and a plasma sampling collection device. The kits of the present invention can also comprise any other component necessary for a kit, such as a container.

Patient plasma can be collected by any known plasma collection device. Some plasma collection devices useful in the present invention include, but are not limited to, vacutainers. The plasma collection devices of the present invention can optionally comprise additives in the device, such as anticoagulants (EDTA, sodium citrate, heparin, oxalate), a gel with intermediate density between blood cells and blood plasma, particles causing the blood to clot, a gel to separate blood cells from serum, thrombin and fluoride, among others.

The kits can also contain additional vessels used for further analysis of the plasma. For example, when the plasma is centrifuged, the centrifuged plasma can be transferred to a vessel, such as a cryostat tube. One of skill in the art will appreciate that other vessels and containers are useful in the present invention.

IV. Examples

Example 1

Determination of Mifepristone Plasma Level

This example provides a procedure for determining the plasma level of mifepristone in a patient.

Three (3) mL of blood was collected from a patient in a vacutainer containing sodium heparin. The blood was centrifuged and the resulting plasma frozen at −70 to −80° C. until assay. For analysis, the plasma samples were warmed and prepared as follows:

1. Using a pipette, 50.0 μL of the sample was aliquoted into a 16×100-mm glass test tube. When a partial volume aliquot was needed, the aliquot was added to the tube and diluted to full volume with blank human plasma.
2. 20.0 μL of the internal standard, mifepristone-d_4 (5.00 m/mL in acetonitrile), was added to the tube, resulting in 2000.0 ng/mL mifepristone-d_4 in plasma.
3. The tube was vortexed for approximately 1 minute.
4. 50.0 μL of 6% ammonium hydroxide was added to the tube.
5. The tube was vortexed for approximately 1 minute.
6. 2.00 mL of MTBE was added to the tube.
7. 2.00 mL of hexane was added to the tube.
8. The tube was vortexed for at least 15 minutes.
9. The tube was centrifuged for at least 10 minutes at 2500 RPM (575×g).
10. The aqueous layer was frozen in a freezer set to maintain −70° C.
11. The upper organic layer was poured into a 13×100-mm polypropylene tube.
12. The organic layer was evaporated in a Turbovap set to 40° C.
13. 200.0 μL of a solution of water:acetonitrile:formic acid (75:25:0.1, v/v/v) was added to the tube.
14. The tube was vortexed for approximately 1 minute.
15. The tube was sonicated for approximately 1 minute.
16. The tube was vortexed for approximately 1 minute.
17. The sample was transferred to a labeled injection vial or well plate.
18. The vial or plate was capped and checked for air bubbles.

The sample was then analyzed by reverse-phase high pressure liquid chromatography using a water:acetonitrile:formic acid (60:40:0.1) mobile phase (isocratic) at a flow rate of 0.3 mL/min. The column was a phenyl column maintained at 50° C. Mifepristone elutes at 4.2 minutes. Following elution, the mobile phase was nebulized using heated nitrogen in a Z-spray source/interface and the ionized compounds detected using a tandem quadrupole mass spectrometer. Mifepristone (molecular weight of 430 g/mol) was detected at m/z 372.30. The internal standard mifepristone-d_4 was detected at m/z 376.30. The ratio of the mifepristone peak height to the mifepristone-d_4 peak height was calculated.

The plasma concentration of mifepristone was then calculated by comparing the experimental ratio to a standard curve of mifepristone:mifepristone-d_4 peak height ratio v. mifepristone concentration. The standard curve was generated by first measuring the mifepristone:mifepristone-d_4 peak height ratios for mifepristone samples at 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL where the mifepristone-d_4 internal standard has a concentration of 2000 ng/mL. The mifepristone:mifepristone-d_4 peak height ratios of these known solutions were then fit to a power equation (Mass Lynx by Micromass, Beverly, Mass.), and the sample with unknown concentrations of mifepristone was compared.

Example 2

Treatment of Cushing's Syndrome

This example provides an open label study of the safety and efficacy in the treatment of Cushing's syndrome. The study was a Phase III trial performed using several investigators at several different sites. The objectives were to demonstrate the efficacy and safety of mifepristone in the treatment of Cushing's syndrome. The number of patients was 50. Patients eligible for randomization were male or nonpregnant female outpatients, and inpatients, if clinically required, with a diagnosis of Cushing's syndrome. Mifepristone was used as the test drug at 300 (1×300 mg tablet), 600 (2×300 mg tablet), 900 (3×300 mg tablet) and 1200 mg (4×300 mg tablet) once a day by mouth. Safety visits occurred at Days 21 and 35. If clinically necessary, a patient was treated as an inpatient. If early termination occurred prior to day 35, the patient returned for a safety follow up visit at day 35.

The primary efficacy endpoint was the proportion of patients with at least a 25% reduction from baseline on a standard two hour oral glucose tolerance test or a 5 month drop in their diastolic blood pressure at study end (6 months). The secondary endpoints were: (1) the patient's global impression; and (2) reduction in previously used antihypertensive medication.

Adverse events, laboratory assessments including electrocardiograms, and physical examination were used to assess safety.

The criteria for assessing study efficacy objective was the proportion of patients with at least a 25% reduction from

baseline on a standard two hour oral glucose tolerance test or a 5 month drop in their diastolic blood pressure at study end (6 months).

TABLE 1

MIFE-C_{trough} (ng/mL) at Each Dose Level Using the Subject Data Set for Responders who Completed the Study

	MIFE-C_{trough} (ng/mL) for Dose of: Dose			
Parameter	300	600	900	1200
N*	30	29	23	14
Mean	1774.28	1951.66	2107.70	2292.86
Median	1455.00	1630.00	1820.00	2022.50
Min	820.00	890.00	992.00	1060.00
Max	4130.00	3850.00	4230.00	5440.00
% CV	46.1	43.2	44.1	47.3
95% CI Lower	1469.15	1631.28	1706.19	1666.13
95% CI Upper	2079.41	2272.04	2509.20	2919.58
Optimal Response (%)	20	20	20	40
Cumulative Optimal Response (%)	20	40	60	100

TABLE 2

Summary of MIFE-C_{trough} Parameters and Prior Dose at First Response Including Early Terminators

	MIFE-ΔC_{trough} from Prior Dose (ng/mL)	MIFE-C_{trough} at Response (ng/mL)
N	26	31
Mean	221.50	1968.68
SD	582.96	834.99
SE	114.33	149.97
Min	−860.00	448.00
Median	250.00	1770.00
Max	2017.00	4210.00
% CV	263.19	42.41
95% CI	235.46	306.28
Lower	−13.96	1662.40
Upper	456.96	2274.95

Example 3

Treatment of Male Patient with Cushing's Syndrome

A 50 year-old male, weighing 175 pounds, presents to physician with Cushing's syndrome. The physician prescribes 300 mg of mifepristone for seven daily doses over a period of seven days. One week later on the day of the seventh daily dose, three (3) mL of blood are collected from the patient and analyzed as described above in the specification. The dose of mifepristone is then adjusted, if necessary, to achieve mifepristone blood levels of greater than 1631 ng/mL. The mifepristone dose can be adjusted a single time to achieve mifepristone blood levels of greater than 1631 ng/mL. Alternatively, several adjustments to the mifepristone dose can be necessary to safely achieve mifepristone blood levels of greater than 1631 ng/mL.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference. Where a conflict exists between the instant application and a reference provided herein, the instant application shall dominate.

What is claimed is:

1. A method for treating a patient suffering from Cushing's syndrome, the method comprising:

A) treating said patient with seven or more daily doses of mifepristone over a period of seven or more days, wherein each of said daily doses contains an initial amount of mifepristone;

B) testing the plasma mifepristone levels of the patient; then

C) if the plasma mifepristone levels of the patient are greater than 1631 ng/mL, continuing to treat the patient with said daily doses of mifepristone; or

D) if the plasma mifepristone levels of the patient are less than or equal to 1631 ng/mL, continuing to treat the patient with daily doses of mifepristone, wherein each daily dose contains an amount of mifepristone that is greater than said initial amount of mifepristone; and

E) repeating step B) and then step C) or step D);

thereby

adjusting the daily dose of the patient to achieve mifepristone plasma levels greater than 1631 ng/mL, with the proviso that the patient is not already suffering from a condition indicated for treatment with mifepristone,

whereby mifepristone treatment optimized to achieve mifepristone plasma levels greater than 1631 ng/mL is provided to the patient suffering from Cushing's syndrome.

2. The method of claim **1**, wherein each of the seven or more daily doses of mifepristone are administered orally.

3. The method of claim **1**, wherein the initial daily dose of mifepristone contains at least 300 mg of mifepristone.

4. The method of claim **1**, wherein the initial daily dose of mifepristone contains at least 600 mg of mifepristone.

5. The method of claim **1**, wherein the initial daily dose of mifepristone contains at least 900 mg of mifepristone.

6. The method of claim **1**, wherein the initial daily dose of mifepristone contains at least 1200 mg of mifepristone.

7. The method of claim **1**, wherein the patient is treated with 28 or more daily doses over a period of 28 or more days.

8. The method of claim **1**, wherein the testing is performed on a sample collected by a plasma sampling collection device and mifepristone levels in the sample are detected using a device suitable for detecting mifepristone plasma levels.

9. The method of claim **1**, wherein mifepristone plasma levels greater than 1631 ng/mL are achieved, wherein the adjusting step comprises further adjusting the daily dose of the patient to maintain mifepristone plasma levels greater than 1631 ng/mL for up to six months.

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